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TREATMENT OF INFLAMMATORY DISORDERS OF THE EPITHELIUM WITH LOW DOSE 2,3-BENZODIAZEPINES

Cross-Reference to Related Applications

The present application is a continuation-in-part of international application PCT/US03/38643 as filed on December 3, 2003 which application claims priority to U.S. patent application No. 10/309,573 as filed on December 3, 2002. The present application claims further benefit from U.S. patent application No. 10/727,940 as filed on December 3, 2003. The disclosures of the PCT/US03/38643 and U.S. patent application Nos. 10/309,573 and 10/727,940 are each incorporated herein by reference.

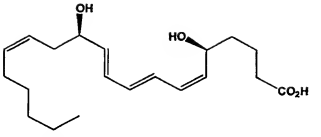
Field of the Invention

The present invention relates to methods of treatment for inflammatory disorders, particularly disorders of epithelial tissue such as that of the skin or gastrointestinal tract.

Background of the Invention

I. Leukotriene B_4 (LTB_4).

Leukotriene B_4 is produced by leukocytes, particularly macrophages and monocytes, upon activation by immune complexes, phagocytosis or other stimuli. In this process, membrane phospholipids are broken down by phospholipase A_2 to release arachidonic acid, which is further metabolized via one of two pathways. The first is via cycloxygenases to produce prostaglandins. The second is via lipoxygenases to form leukotriene A_4 (LTA_4). LTA_4 is converted to LTB_4 or LTC_4. LTB_4 is a potent chemotactic agent that stimulates neutrophil and macrophage migration (chemotaxis) to sites of inflammation. The structure of LTB_4 is shown below.



The known pathophysiological responses of LTB_4 include: induction of potent neutrophil chemotactic activity, promotion of adhesion of polymorphonuclear leukocytes (PMN's) to vasculature, increase in vascular permeability, stimulation of the release of lysosomal enzymes, by PMN's. The pro-inflammatory action of LTB_4 has been demonstrated *in vivo*, wherein topical LTB_4 on human skin promotes the infiltration of PMN's and other inflammatory cells. Intradermal injection of LTB_4 induces accumulation of neutrophils at the injection site. Intravenous injection of LTB_4 causes rapid but transient neutropenia (Kingsbury *et al.*, *J. Med. Chem.*, 1993, 36, 3308-3320; and references cited therein).

In addition, the presence of physiologically relevant LTB_4 concentration at inflammatory sites has been associated with, for example, disease states such as psoriasis, asthma and active gout; in colonic mucosa associated with inflammatory bowel disease; in synovial fluid from patients with active rheumatoid arthritis (RA); and in reperfusion injury. All of these observations together support the involvement of LTB_4 in human inflammatory disease (Kingsbury *et al*, and Griffeths *et al.*, *Proc. Natl. Acad. Sci.*Vol. 92, pp517-521, Jan. 1995; and references cited therein.).

LTB_4 is believed to interact with two sub-groups of receptor: a high-affinity receptor and a low-affinity receptor. Research indicates that the high-affinity receptor mediates chemotaxis and that the low-affinity receptor mediates LTB_4-induced secretory and oxidase-activation responses (Yokomizo *et al.* 2000). Some LTB_4 antagonists are observed to antagonize all LTB_4 mediated activity. Other LTB_4 antagonists modulate only the activity associated with one but not the other sub-population of LTB_4 receptors.

I. LTB_4 Antagonists.

Compounds, which act as antagonists of LTB_4 include, for example: structural analogs of LTB_4 such as LTB_4-dimethyl amide and 20-CF_3-LTB_4; SM-9064; U-75302; Ly-223982; SC-41930; ONO 4057 (Prostaglandins, 44(4):261-275, 1992); RG-14893; (*E*)-3-[2-[6-[3-(3-butoxyphenyl)-3-hydroxypropenyl]pyridin-2-yl]-1-hydroxyethyl]benzoate-benzoic acid, lithium salt (Kingsbury J. Med. Chem., 1993, 36, 3308-3320, and references cited therein); the natural product Leucettamine A and a structural analog, 1-methyl-2-amino-4-[[4'-[4"-(hydroxybutyl)phenyl]methyl]-5-(phenyl-methyl)imidazole (Boehm *et al, J. Med. Chem.*, 1993, 36, 22, 3333-3340); a series of pyridine-2-acrylic acids (Kingsbury *et al., J. Med. Chem.*, 1993, 36, 22, 3321-3332); SC-45694 (Tsai *et al, J. Pharm. Exp. Ther.*, 268, 3, 1493-1498); a series of essential fatty acids (Yagaloff *et al., Prostaglandins, Leukotrienes and Essential Fatty Acids* (1995), 52, 293-297); and FPL 55712 and FPL 55231 (Cheng *et al., J. Pharm. Exp. Ther.*, 236(1), 1985). The structures of these compounds show many similarities to the structure of LTB_4.

III. 2,3-Benzodiazepines.

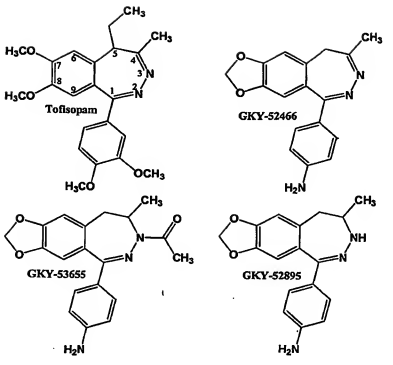
Certain 2,3-benzodiazepines have been explored extensively for their potent CNS modulating activity. Compounds such as tofisopam (Grandaxin®), girisopam, and norisopam have demonstrated substantial anxiolytic and antipsychotic activity.

Tofisopam has been shown in humans to have an activity profile that is significantly different from that of widely used 1,4-benzodiazepine (BZ) anxiolytics such as diazepam (Valium®) and chlordiazepepoxide (Librium®). The 1,4-benzodiazepine, in addition to having sedative-hypnotic activity, also possess muscle relaxant and anticonvulsant properties which, though therapeutically useful in some disease states, are nonetheless potentially untoward side effects. Thus, the 1,4-benzodiazepines, though safe when administered alone, may be dangerous in combination with other CNS drugs including alcohol.

Tofisopam, in contrast, is a non-sedative anxiolytic that has no appreciable sedative, muscle relaxant or anticonvulsant properties (Horvath *et al., Progress in Neurobiology,* 60 (2000), 309-342). In clinical studies, tofisopam improved rather than impaired psychomotor performance and showed no interaction with ethanol (*Id.*). These observations comport with data that show that tofisopam does not interact with central BZ receptors and binds only weakly to peripheral BZ receptors. Additional studies have shown that tofisopam enhances mitogen-induced lymphocyte proliferation and IL-2 production *in vitro*.

Other 2,3-benzodiazepines that are structurally similar to tofisopam have been investigated and shown to have varying activity profiles. For example, GYKI-52466 and GYKI-53655 (structures shown below) act as noncompetitive glutamate antagonists at the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) site, and have demonstrated neuroprotective, muscle relaxant and anticonvulsant activity (*Id.*). Another group of 2,3-benzodiazepines that have been investigated are represented by the compound GYKI-52895, and show activity as selective dopamine uptake inhibitors with potential use in antidepressant and anti-Parkinsonism therapy.

Tofisopam (structure shown below), with the atom numbering system indicated) is a racemic mixture of (*R*)- and (*S*)- enantiomers. This is due to the asymmetric carbon, *i.e.*, a carbon with four different groups attached, at the 5-position of the benzodiazepine ring.



The molecular structure and conformational properties of tofisopam have been determined by NMR, CD and X-ray crystallography (Visy *et al.*, *Chirality* 1:271-275 (1989)). The 2,3-diazepine ring exists as two different conformers. The major tofisopam conformers, (+)*R* and (-)*S*, contain a 5-ethyl group in a quasi-equatorial position. The 5-ethyl group is positioned quasi-axially in the minor conformers, (-)*R* and (+)*S*. Thus, racemic tofisopam can exist as four molecular species, *i.e.*, two enantiomers, each of which exists as two conformations. The sign of the optical rotation is reversed upon inversion of the diazepine ring from one conformer to the other. In crystal form, tofisopam exists only as the major conformations, with dextrorotatory tofisopam being of the (*R*) absolute configuration. (Toth *et al.*, *J. Heterocyclic Chem.*, 20:709-713 (1983); Fogassy *et al.*, *Bioorganic Heterocycles*, Van der Plas, H.C., Ötvös, L, Simongi, M., eds. Budapest Amsterdam: Akademia; Kiado-Elsevier, 229:233 (1984)).

Differential binding of the (+) and (-) conformations of tofisopam has been reported in binding studies with human albumin (Simongi *et al. Biochem. Pharm.*, 32(12), 1917-1920, 1983). The two (+/-) conformers have also been reported as existing in equilibrium (Zsila *et al.*, *Journal of Liquid*

Chromatography & Related Technologies, 22(5), 713-719, 1999; and references therein).

The optically pure (*R*)-enantiomer of tofisopam (*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine) has been isolated and shown to possess the nonsedative anxiolytic activity of the racemic mixture. See US Patent 6,080,736; the entire disclosure of which is incorporated herein by reference.

IV. Inflammatory Disorders.

Frequently, inflammatory disorders occur at or near an epithelium, such as that of the skin, the cornea, or the gastrointestinal lining.

A. Inflammatory Bowel Disease

Crohn's disease (CD) and ulcerative colitis (UC), and, to a lesser extent, indeterminate colitis and infectious colitis, are collectively referred to as inflammatory bowel disease IBD. Inflammatory bowel diseases are chronic recurrent inflammatory diseases of unclear etiology, affecting the small intestine and colon. IBD can involve either or both the small and large bowel. These disorders fall into the category of "idiopathic" IBD because the etiology for them is unknown.

Pathologic findings are generally not specific, although they may suggest a particular form of IBD. "Active" IBD is characterized by acute inflammation. "Chronic" IBD is characterized by architectural changes of crypt distortion and scarring. The term "crypt" refers to a deep pit that protrudes down into the connective tissue surrounding the small intestine. Crypt abscesses (active IBD characterized by the presence of neutrophils in crypt lumens) can occur in many forms of IBD, not just UC. Under normal conditions the epithelium at the base of the crypt is the site of stem cell proliferation and the differentiated cells move upwards and are shed 3-5 days later at the tips of the villi. This normal process, necessary for proper bowel function, is interrupted by IBD.

UC involves the colon as a diffuse mucosal disease with distal predominance. The rectum is virtually always involved, and additional portions of colon may be involved extending proximally from the rectum in a continuous

pattern. Most often UC occurs in young people 15 to 40 years of age. UC occurs only in the inner lining of the colon (large intestine) or rectum. When it is localized in the rectum, it is called "proctitis".

CD is a chronic inflammatory disease that has periods of remission (time when person feels well) and relapse (when a person feels ill). CD is an inflammation and ulceration process that occurs in the deep layers of the intestinal wall. The most common areas affected are the lower part of the small intestine, called the ileum, and the first part of the colon. This type of CD is called ileocolitis. CD can infrequently affect any part of the upper gastrointestinal tract. Aphthous ulcers, which are similar to cold sores, are common. Ulcers can also occur in the esophagus, stomach and duodenum.

Therapy for IBD has historically included administration of corticosteroids. However drawbacks of long term corticosteroid therapy include masking (or induction) of intestinal perforation, osteonecrosis and metabolic bone disease. Additional problems relate to development of corticosteroid dependency (Habnauer, *New England Journal of Medicine*, 334(13), p 841-848, 1996). Aminosalicylates such as sulfasalazine and mesalamine have been used to treat mild or moderately active UC and CD, and to maintain remission (*Id* at 843). Immunomodulatory drugs such as azathioprine and mercaptopurine have been used in long term treatment for patients with IBD. Common complications with both of these drugs include pancreatitis, which occurs with an incidence of 3-15% of patients, and bone marrow suppression, which requires regular monitoring. More potent immunosuppressive drugs such as cyclosporine and methotrexate have been employed, but toxicity of these drugs limits their use to specific situations of refractory disease states. Other therapeutic approaches include antibiotic therapy and nutritional therapy. Often, therapy involves a combination of the above-described drug therapies in addition to surgical resection of the bowel.

There is no cure for IBD. Ultimately, the chronic and progressive nature of IBD demands a long-term treatment that maximizes the local

antiinflammatory effect while minimizing the global systemic effect on the immune system.

Chronic inflammatory disorders such as CD typically demonstrate periods of remission between intervals when the inflammatory is active and requires acute treatment. This is an example of a circumstance wherein it is known beforehand that an individual will develop, or is likely to develop an inflammatory disorder.

Irritable bowel syndrome (IBS) is a disorder of the bowel which is distinct from IBD. IBS affects at least 10% to 20% of adults in the U.S. IBS is the most common disorder diagnosed by gastroenterologists and one of the top ten most frequently diagnosed conditions among U.S. physicians.

IBS is classified as a "functional gastrointestinal disorder," which means there is a disturbance in bowel function. IBS is not a considered a disease, but rather a syndrome, *i.e.*, a group of symptoms. The symptoms typically include chronic abdominal pain/discomfort, and irregular bowel function, *e.g.*, diarrhea, constipation, or alternating diarrhea and constipation.

Unlike IBD, IBS does not cause inflammation. IBS sufferers show no sign of disease or abnormalities on examination of the colon. Thus, though IBD and IBS share some similar symptoms, particularly cramping and diarrhea, the underlying disease process is quite different. IBD involves inflammation or destruction of the bowel wall, which can lead to deep ulcerations and narrowing of the intestines. IBS is a disorder of the gastrointestinal (GI) tract for which no apparent cause can be found. An individual can simultaneously have both IBS and an inflammatory disorder such as IBD. When this occurs, imprecise diagnosis may lead to inadequate medical intervention.

B. Inflammatory Skin Disorders:

1. Psoriasis.

Another chronic inflammatory condition believed to be mediated by LTB_4 is psoriasis. Psoriasis is a chronic, recurrent, papulosquamous plaque on areas of trauma such as the elbow, knee or scalp, though it may appear elsewhere on the skin. Psoriasis may coexist with *lupus erythematosis* in some

individuals. Current treatments include topical administration of psoralens. "Psoralens" refers to a group of substances found in many different plants; especially *psoralea corylifolia*. Psoralens interact with nucleic acids and are also used as research tools. Psoriasis is also treated by long-wave ultraviolet radiation. Neither treatment cures or prevents recurrence of psoriasis symptoms.

2. Atopic Dermatitis/Eczema.

Atopic dermatitis is a chronic disease that affects the skin. In atopic dermatitis, the skin becomes extremely itchy. Scratching leads to redness, swelling, cracking, "weeping" clear fluid, and finally, crusting and scaling. In most cases, there are periods of exacerbations followed by periods of remissions. Although it is difficult to identify exactly how many people are affected by atopic dermatitis, an estimated 20% of infants and young children experience symptoms of the disease. Approximately 60% of these infants continue to have one or more symptoms of atopic dermatitis in adulthood. Thus, more than 15 million people in the United States have symptoms of the disease

3. Contact Dermatitis.

Contact dermatitis is a reaction that occurs when the skin comes into contact with an allergen, *i.e.*, a substance to which the body is allergic. Allergens, though harmless to most individuals, cause an allergic reaction in individuals having a congenital or acquired hypersensitivity to the specific allergen.

C. Rheumatoid Arthritis (RA).

Another chronic inflammatory disorder believed to be mediated by LTB_4 is RA, which is an autoimmune disease of the joints. RA is characterized by the following criteria 1-7, wherein criteria 1-4 are present for more than 6 weeks: (1) morning stiffness in and around joints lasting at least one hour before maximum improvement; (2) soft tissue swelling (arthritis) of three or more joints observed by a physician; (3) swelling (arthritis) of the proximal interphalangeal, metacarpal phalangeal, or wrist joints; (4) symmetric swelling; (5) rheumatoid nodules, *i.e.*, a granulomatous lesion characterized by central

necrosis encircled by a palisade of monocytes and an exterior mantle of lymphocytic infiltrate. These lesions present as subcutaneous nodules, especially at pressure points such as the elbow in individuals with RA or other rheumatoid disorders; (6) presence of rheumatoid factors, *i.e.*, an autoantibody in the serum of individuals with RA; and (7) roentgenographic erosions, *i.e.*, joint lesions visible on an X-ray.

RA is a chronic disorder for which there is no known cure. The major goals of treatment of RA are to reduce pain and discomfort, prevent deformities and loss of joint function, and maintain a productive and active life. Inflammation must be suppressed and mechanical and structural abnormalities corrected or compensated by assistive devices. Treatment options include reduction of joint stress, physical and occupational therapy, drug therapy, and surgical intervention.

There are three general classes of drugs commonly used in the treatment of RA: non-steroidal anti-inflammatory agents (NSAID's), corticosteroids, and remittive agents or disease modifying anti-rheumatic drugs (DMARD's). NSAID's and corticosteroids have a short onset of action while DMARD's can take several weeks or months to demonstrate a clinical effect. DMARD's include leflunomide (Arava™), etanercept (Enbrel™), infliximab (Remicade™), antimalarials, methotrexate, gold salts, sulfasalazine, d-penicillamine, cyclosporin A, cyclophosphamide and azathioprine. Because cartilage damage and bony erosions frequently occur within the first two years, rheumatologists now move more aggressively to a DMARD agent.

Treatment of RA by chronic administration of a corticosteroid involves the same side effect profile as discussed regarding IBD above. Chronic administration of NSAID's also produces side effects. The most common toxicity of NSAID's is gastrointestinal disturbance. Because prostaglandins play a role in the regulation of renal blood flow and maintenance of glomerular filtration, NSAID's can impair renal function in certain patients. Weight gain and cushingoid appearance is a frequent problem and source of patient complaints. Recent studies have raised concern over the increased

cardiovascular risk and accelerated osteoporosis associated with low dose prednisone particularly at doses above 10 mg daily.

D. Gout.

Gout is another inflammatory disorder believed to be mediated by LTB_4. Gout is characterized by a disturbance of uric-acid metabolism occurring chiefly in males. Gout is characterized by painful inflammation of the joints, especially of the feet and hands, and arthritic attacks resulting from elevated levels of uric acid in the blood and the deposition of urate crystals around the joints. The condition can become chronic and result in deformity.

Gout can present another circumstance wherein it is known beforehand that an individual will or is likely to develop an inflammatory disorder. In the instance of patients undergoing radiotherapy or chemotherapy, the individual may experience a dramatic rise in serum uric acid levels associated with lysis of the tumor mass. Such large increases in uric acid can deposit urate crystals in synovial fluid of joints thereby causing the inflammatory disorder, gout. When such a rise in serum uric acid levels is known to be likely, prophylaxis with an LTB_4 antagonist can act to prevent the inflammatory condition of gout.

E. Radiation–induced Gastrointestinal Inflammation.

Radiation–induced gastrointestinal inflammation is another inflammatory disorder believed to be mediated by LTB_4. Radiation works by damaging cancer cells, but unfortunately can damage non-diseased tissue as well, causing a typical inflammatory reaction in response. Therapeutic radiation is thus generally applied to a defined area of the subject's body which contains abnormal proliferative tissue in order to maximize the dose absorbed by the abnormal tissue and minimize the dose absorbed by the nearby normal tissue. However, it is difficult (if not impossible) to selectively administer therapeutic ionizing radiation to the abnormal tissue. Thus, normal tissue proximate to the abnormal tissue is also exposed to potentially damaging doses of ionizing radiation throughout the course of treatment. Moreover, some treatments that require exposure of the subject's entire body to the radiation, in a procedure called "total body irradiation", or "TBI." The efficacy of radiotherapeutic

techniques in destroying abnormal proliferative cells is therefore necessarily balanced by the associated cytotoxic effects on nearby normal cells.

After or during a course of radiotherapy, LTB_4-mediated inflammatory processes may be triggered, causing damage to the bowel, and leading to sloughing of the cells of the inner lining of the GI tract. Radiation–induced gastrointestinal inflammation can present another circumstance wherein it is known beforehand that an individual will or is likely to develop an inflammatory disorder. In the instance of patients undergoing radiotherapy, the inflammation, damage and sloughing of the gastrointestinal tract is a predictable side effect of the radiotherapy.

F. Mucositis.

Mucositis involves ulcerative breakdown of mucosal epithelial tissue, and is literally defined as inflammation of the mucous membrane. The pathophysiology of mucositis in response to toxic insults to the mucosa by chemotherapy or by ionizing radiation is complex and involves a cascade of interactions among cells, cytokines and the oral microflora. The underlying premise for susceptibility of the mucosa of the oropharynx and gastrointestinal tract to chemotherapy or radiation damage is related to rapid epithelial stem cell turnover. Mucositis may be characterized by the following phases:

1. Early inflammatory phase characterized by release of inflammatory cytokines in response to local tissue damage caused by cytotoxic agent(s);

2. Epithelial phase characterized by death of basal cells, which hinders re-population of the epithelium. This inability to regenerate leads to atrophy followed by ulceration. The ulceration represents loss of an important anatomic barrier at a site of local microflora;

3. Infection phase characterized by local invasion of microflora that results in an inflammatory response to the local infection. The inflammation results in additional local tissue damage and possibly erosive ulceration; and

4. Healing phase characterized by resolution of the infection and regeneration of epithelium.

Oral mucositis produces the following clinical symptoms and signs resulting from cellular damage: 1) sensation of dryness; 2) asymptomatic redness and erythema; 3) solitary white elevated desquamative patches which are painful upon pressure contact; and 4) large, painful, contiguous pseudomembranous lesions associated with dysphagia and decreased oral intake. These spontaneously painful lesions histopathologically show loss of epithelial cells to the basement membrane, which exposes the connective tissue stroma with its associated innervation.

As with oral mucosa, gastrointestinal mucosal damage results from disturbance of cellular mitosis that leads to reduction in the turnover rate of the basal cells of the intestinal crypts. The symptoms and signs of gastrointestinal mucositis include tenesmus (painful ineffectual straining at stool), pain, bleeding, diarrhea, telangectasia (neovascularization), and progression to ulceration. Early signs of diarrhea include increased stool frequency, loose or watery stool, food aversion, increased bowel sounds, abdominal pain, and some loss of skin turgor indicative of dehydration. When the diarrhea is severe it may be associated with mucosal ulceration, bleeding, intestinal perforation and proctitis. Stool examination may reveal occult blood and fecal leukocytes.

G. Necrotizing Enterocolitis.

Necrotizing enterocolitis is an inflammatory disease of unknown etiology that afflicts between 1-5% of all infants admitted to neonatal intensive care units, most of whom are premature infants. Signs and symptoms include abdominal distention, gastrointestinal hemorrhage, and feeding intolerance. The disease most often involves the ileum and colon, and is characterized by loss of epithelium and submucosal edema, ulcerations, and, in severe cases, transmural necrosis.

H. Aphthous Ulcers (Oral).

Although the cause of aphthous ulcers remains unknown, many physicians believe they are caused by autoimmune phenomena, which cause the destruction of discrete areas of the oral mucosa which leads to oral ulceration.

Among the cytokines present in these active areas of ulceration, TNF-α appears to play a predominant role.

I. Gingivitis/Periodontitis.

Adult periodontitis is strongly associated with infection by Porphyromonas gingivalis. Proteolytic enzymes, which are produced in large quantity by this bacteria, are considered as important pathogenic agents. The increased production and flow of gingival crevicular fluid (GCF) is an important change in gingival tissues during periodontal infection, correlating with clinical indices of gingival inflammation. Salivary protein and albumin concentrations of individuals with periodontitis, which are an indication of plasma leakage due to vascular permeability enhancement (VPE), are significantly increased compared to healthy subjects. The production of GCF appears dependent on VPE induced at periodontitis sites, presumably involving proteinase(s) of P. gingivalis in their generation.

J. Esophagitis.

The most common cause of esophagitis is the chronic reflux of hydrochloric acid from the stomach due to inefficiency of the cardiac sphincter of the stomach. The chronic presence of acid in the lower esophagus leads to damage of the esophageal mucosa. In the most severe form, a syndrome called Barrett's esophagus can develop which often leads to esophageal cancer. Other causes of esophagitis include parenteral chemotherapy and ionizing radiation, associated with radiation therapy for cancer in the thoracic cavity.

K. Pharyngitis.

Pharyngitis is defined as an infection or irritation of the pharynx and/or tonsils. The etiology is usually infectious, with 40-60% of cases being of viral origin and 5-40% of cases being of bacterial origin. Other causes include allergy, trauma, toxins, and neoplasia. It has been estimated that children in the US experience more than five upper respiratory infections (URIs) per year and an average of one streptococcal infection every four years. The occurrence in adults is about one half that rate. The most significant bacterial agent causing

pharyngitis in both adults and children is GABHS infection (Streptococcus pyogenes), and the most significant viruses are rhinovirus and adenovirus.

L. Ocular Diseases.

1. Retinitis.

Inflammation of the light sensitive retina, retinitis, can occur due to a variety of viral, bacterial or autoimmune etiologies. The end result is destruction of the retina and loss of sight.

2. Uveitis.

Inflammation of the anterior portion of the eye and/or its associated structures, the iris and cornea occurs with a relatively high frequency in patients with autoimmune disorders.

3. Conjunctivitis.

Conjunctivitis is an inflammation of the conjunctivae, which are the mucous membranes covering the white of the eyes and the inner side of the eyelids. There are five major types of conjunctivitis. (1) Bacterial conjunctivitis is an infection caused by bacteria. (2) Viral conjunctivitis may be caused by a virus called 'adenovirus'. (3) Chlamydial conjunctivitis is caused by an organism called Chlamydia trachomatis. (4) Allergic conjunctivitis is common in people who have other signs of allergic disease such as hay fever, asthma and eczema. (5) Reactive conjunctivitis (chemical or irritant conjunctivitis) is caused by a chemical irritant, *e.g.*, chemicals in swimming pools, smoke or solvent fumes.

M. Peptic Ulcer Disease.

Inhibition of gastric acid secretion with H_2-receptor antagonists and, more recently, blockers of H^+, K^+-ATPase (also known as the proton pump) has been the mainstay of therapy for peptic ulcer disease. The pathophysiology of peptic ulcers remains obscure. An appreciation of the complexity of the physiology of the gastric mucosa has led to a hypothesis that peptic ulcers are the result of an imbalance in the relative importance of aggressive (acid, pepsin) and protective (mucus, bicarbonate, blood flow, prostaglandins, etc.) factors. Infection of the mucosa of the human gastric antrum with the bacterium

Helicobacter pylori has been widely accepted as the cause of chronic, active, type B gastritis. Further, this form of gastritis has been linked directly to peptic ulcer disease by studies showing that eradication of H. pylori reverses this gastritis and prevents duodenal ulcer relapse.

V. Agents Useful in Treatment of Inflammatory Disorders.

Numerous chemical entities have been investigated for biological activity as antiinflammatory agents. Particular classes of compounds which have been investigated include aminosalicylates, corticosteroids, antimetabolites, immunosuppressants, tumor necrosis factor alpha (TNF-α) inhibitors, inhibitors of leukotriene synthesis *e.g.*, 5-lipoxygenase (5-LO) inhibitors, and leukotriene antagonists.

Exemplary compounds of interest that have been shown to possess activity in models of inflammatory disorders are listed in Table 1 along with putative mechanisms of action and the indications for which they have been investigated.

Table 1

Agent	Indication or potential indication	Mechanism of action
ETH615 (4-[2-quinolylmethoxy]-N-[3-flurobenzyl]-phenyl-aminomethyl-4-benzoic acid	dermatitis	5-LO inhibitor Inhibits inflammation-induced chemotaxis
15-HETE (15-hydroxyeicosa-tetraenoic acid)	psoriasis	Inhibits LTB_4 synthesis Inhibits LTB_4-induced chemotaxis
Leflunomide (Arava)	asthma (marketed)	Inhibits pyrimidine synthesis Inhibits LTB_4 release Inhibits inflammation-induced chemotaxis Inhibits proliferation of epidermal keratinocytes
Linetastine		5-LO inhibitor
Lonapalene (RS 43179)	psoriasis	5-LO inhibitor
R-68,151	psoriasis	5-LO inhibitor
MK 886 3-[1-(p-Chloro-benzyl)-5-(isopropyl)-3-tert-butylthio-indol-2-yl]-2, 2- dimethyl-propanoic acid	asthma	Inhibits 5-LO activation protein

Agent	Indication or potential indication	Mechanism of action
Zileuton (Zyflo)	asthma (marketed)	5-LO inhibitor
SC41930 7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxy]-8-propylchromane-2-carboxylic acid	psoriasis, IBD, RA	LTB_4 receptor antagonist
SC50605		LTB_4 receptor antagonist
SC53228 ((+)-(S)-7-[3-{2-Cyclo-propylmethyl}-3-methoxy-4-{(methylamino)carbonyl} phenoxy]propoxy)-3,4-dihydro-8-propyl-2H-1-benzopyran-2-propanoic acid	psoriasis, UC, dermatitis	LTB_4 receptor antagonist
SC52798	Inflammation, Psoriasis, UC	LTB_4 receptor antagonist
CGS-25019C 4-(5-[4-{Aminoiminomethyl} phenoxy]-pentoxy)-3-methoxy-N,N-bis(1-methyl-ethyl)-benzamide-(Z)-2-butenedioate	asthma, bronchitis, RA	LTB_4 receptor antagonist
ONO-4057 5-{3-[(5E)-6-(4-methoxy-phenyl)hex-5-enyloxy]-2-(2-carboxyethyl)phenoxy}pentanoic acid	psoriasis, UC, Behcet's	LTB_4 receptor antagonist
SB-201993	psoriasis, RA	LTB_4 receptor antagonist
SB-209247 (E-3-(6-[{(2,6-Dichloro-phenyl)-thio}-methyl]-3-[2-phenyl-ethoxy]-2-pyridinyl)-2-propenoic acid	psoriasis, eczema	LTB_4 receptor antagonist
VML295 (LY293111) (2-[2-Propyl-3-{2-ethyl-4-(4-fluoro-phenyl)-5-hydroxy-phenyl}propoxy]phenoxy)benzoic acid	psoriasis, asthma, UC, RA, inflammation	LTB_4 receptor antagonist
CP-105696 1-{(3S,4R)-4-hydroxy-3-[(4-phenylphenyl)-methyl]-chroman-7-yl}cyclo-pentane-carboxylic acid	RA, asthma, rejection	LTB_4 receptor antagonist

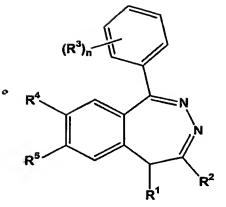
Agent	Indication or potential indication	Mechanism of action
CP-195543 (+)-2-(3-Benzyl-4-hydroxy-chroman-7-yl)-4-trifluoro-methylbenzoic acid	RA, inflammation	LTB_4 receptor antagonist
BIIL 284	cystic fibrosis, asthma	LTB_4 receptor antagonist
U-75302 6-[6-((5Z,1E)-3-hydroxy-undeca-1,5-dienyl)-2-pyridyl]-hexane-1,5-diol	asthma	LTB_4 receptor antagonist
LY 255283	asthma	LTB_4 receptor antagonist
VM 301	wound healing, dermatitis, inflammation	LTB_4 receptor antagonist
ZK 158252	psoriasis	LTB_4 receptor antagonist

New antiinflammatory agents are needed which are useful in the treatment of inflammatory disorders such as IBD, RA, gout, psoriasis and radiation-induced gastrointestinal inflammation. In particular, agents are needed that are appropriate for chronic long-term use in treatment. In addition, agents are needed that are useful in the prevention of inflammatory disorders, particularly LTB_4-mediated inflammatory disorders that occur secondary to observable events such as ionizing radiation therapy.

Summary of the Invention

In one embodiment of the invention there is provided a method of treatment or prevention of inflammatory disorders in an individual, particularly, inflammatory disorders affecting epithelial tissues, in particular, IBD, including CD and, UC, psoriasis; rheumatoid arthritis; gout and radiation-induced gastrointestinal inflammation.

The method comprises administering to the individual an effective amount of at least one compound according to formula I:



I

wherein:

R^1 is -(C_1-C_7)hydrocarbyl or -(C_2-C_6)heteroalkyl;

R^2 is selected from the group consisting of –H, and -(C_1-C_7)hydrocarbyl, wherein R^1 and R^2 may combine to form a carbocyclic or heterocyclic 5- or 6-membered ring;

R^3 is independently selected from the group consisting of –O(C_1-C_6)alkyl, -OH, -O-acyl, -SH, –S(C_1-C_3)alkyl, –NH_2, -NH(C_1-C_6)alkyl, -N((C_1-C_6)alkyl)$_2$, -NH-acyl, -NO_2 and halogen;

n is 1, 2 or 3;

R^4 and R^5 are independently selected from the group consisting of –O(C_1-C_6)alkyl, -OH, O-acyl, -SH, –S(C_1-C_3)alkyl, –NH_2, NH-acyl and halogen, wherein R^4 and R^5 may combine to form a 5, 6 or 7-membered heterocyclic ring; ora pharmaceutically-acceptable salt of such a compound, wherein the compound is administered at a dose of less than about 50 mg/day. Preferably the compound is administered at a dose of less than about 25 mg/day, more preferably less than about10 mg/day and even more preferably at a dose of less than about 1 mg/day. Preferred liquid formulations are administered at a dose of less than about 10 mg/ml and more preferably at a dose of less than about 1 mg/ml. According to one embodiment of the invention, there is provided a method of treatment of inflammatory disorders of epithelial tissue, wherein said inflammatory disorder is a skin disorder. In another embodiment, the inflammatory disorder of the epithelial tissue is a gastrointestinal disorder. According to another embodiment of the invention, the administered compounds

according to formula I comprise a racemic mixture of compounds with respect to the absolute conformation at the 5-position of the benzodiazepine ring.

According to a preferred embodiment of the invention, the administered compounds according to formula I comprise an (*R*)-enantiomer, substantially free of the corresponding (*S*)-enantiomer of the same compound, with respect to the absolute conformation at the 5-position of the benzodiazepine ring,

Preferably, the administered compound of formula I comprising an (*R*)-enantiomer, or a pharmaceutically-acceptable salt of such a compound, comprises 85% or more by weight of the (*R*)-enantiomer. More preferably, the administered compound of formula I comprising an (*R*)-enantiomer, or a pharmaceutically-acceptable salt of such a compound, comprises 90% or more by weight of the (*R*)-enantiomer. Even more preferably, the administered compound of formula I comprising an (*R*)-enantiomer, or a pharmaceutically-acceptable salt of such a compound, comprises 95% or more by weight of the (*R*)-enantiomer. Most preferably, the administered compound, comprising an (*R*)-enantiomer of formula I, or a pharmaceutically-acceptable salt of such a compound, comprises 99% or more by weight of the (*R*)-enantiomer.

According to one embodiment of the invention:

R^1 is -(C_1-C_6)alkyl;

R^2 is selected from the group consisting of –H and -(C_1-C_6)alkyl;

R^3 is independently selected from the group consisting of –O(C_1-C_6)alkyl, -O-acyl and -OH;

n is 1, 2 or 3; and

R^4 and R^5 are independently selected from –O(C_1-C_6)alkyl, -O-acyl and -OH, wherein, R^4 and R^5 may combine to form a 5, 6 or 7-membered heterocyclic ring; or a pharmaceutically-acceptable salt of such a compound.

According to a preferred embodiment of the invention:

R^1 is $–CH_2CH_3$;

R^2 is -(C_1-C_6)alkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of –OH and $-OCH_3$; and

n is 1, 2 or 3;

or a pharmaceutically-acceptable salt of such a compound.

According to a further preferred embodiment of the invention:

R^1 is $-CH_2CH_3$;

R^2 is $-CH_3$;

R^3, R^4 and R^5 are independently selected from the group consisting of –OH and $-OCH_3$; and

n is 1, 2 or 3; or a pharmaceutically-acceptable salt of such a compound.

According to a further preferred embodiment of the invention:

R^1 is $-CH_2CH_3$;

R^2 is $-CH_3$;

R^3, R^4 and R^5 are independently selected from the group consisting of –OH and $-OCH_3$; and

n is 2 ; or a pharmaceutically-acceptable salt of such a compound.

According to a further preferred embodiment of the invention:

R^1 is $-CH_2CH_3$;

R^2 is $-CH_3$;

R^3, R^4 and R^5 are independently selected from the group consisting of –OH and $-OCH_3$;

n is 2; and wherein R^3 comprises substituents at the 3- and 4- positions of the phenyl ring; or a pharmaceutically-acceptable salt of such a compound.

Preferred racemic compounds according to formula I, for administration, are selected from the group consisting of:

1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine;

1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

1-(3-methoxy-4-hydroxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine;

1-(3-methoxy-4-hydroxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine;

1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine; and

pharmaceutically-acceptable salts of such compounds.

More preferably, the compound according to formula I, for administration is 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine; or

a pharmaceutically-acceptable salt thereof.

Preferred compounds comprising (*R*)-enantiomers according to formula I, for administration, are selected from the group consisting of:

(*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

(*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine;

(*R*)-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

(*R*)-1-(3-methoxy-4-hydroxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine;

(*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine;

(*R*)-1-(3-methoxy-4-hydroxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine; and

(*R*)-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine;

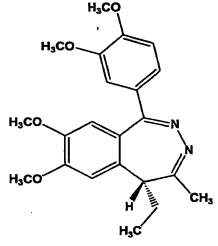
substantially free of the corresponding (*S*)-enantiomers,

and pharmaceutically-acceptable salts of such compounds.

Most preferably, the compound according to formula I, for administration is (*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-

5H-2,3-benzodiazepine, substantially free of the corresponding (*S*)-enantiomer; or a pharmaceutically-acceptable salt thereof.

The compound, (*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine, the (*R*)-enantiomer of tofisopam, is shown in the structure diagram below.



(R)-tofisopam

According to another aspect of the invention, the aforesaid compounds are used in the preparation of medicaments for treating or preventing an inflammatory disorder.

According to another embodiment of the invention there is provided a method of treatment or prevention of inflammatory disorders in an individual, particularly, inflammatory disorders of the epithelium comprising administering to the individual an effective amount of at least one compound according to formula I as defined herein in combination with at least one additional therapeutic agent. Preferably the additional therapeutic agent is selected from the group consisting of aminosalicylates, corticosteroids, antimetabolites, immunosuppressants, TNF-α inhibitors, 5-LO inhibitors and leukotriene antagonists, wherein the leukotriene antagonist is not a compound of formula I.

The invention also encompasses a method of addressing one or a combination of inflammatory disorders of epithelial tissue in a mammal in which an unexpectedly low amount of the compound can be used. More specifically, it has been found that it is possible to achieve good anti-inflammatory activity when the compound is contacted directly on the skin (i.e.,

adminstered topically). Preferred administration routes generally include contacting the epithelium with a formulation in which the compound is present in an amount well below about 100 mg/ml, for example, about 50 mg/ml or well below 10 mg/ml, for example 1 mg/ml or 0.1mg/ml. For skin or for the cornea, a preferred formulation is a topical formulation.

It is believed that the compounds of Formula I are particularly useful to address one or a combination of gastrointestinal disorders in a mammal such as a human patient. Thus according to one embodiment, the invention features a method for preventing, treating, reducing the severity of, or delaying onset of a gastrointestinal disorder that includes administering to the patient an effective amount of the compound. The compound can be used alone as the sole active agent or in combination with one, a few or several other agents including other compounds according to Formula I. Preferred administration routes include those generally well suited for gastrointestinal delivery such as intracolonic delivery (eg an enema or suppository) and other delivery methods described below.

Definitions

The term "inflammation" or inflammatory response" refers to a defense reaction of living tissue to injury. The response serves to contain and to repair the injury. Multiple chemical mediators of inflammation derived from either plasma or cells have been observed. Compounds produced in the metabolism of arachidonic acid, such as prostaglandins and leukotrienes, also affect inflammation, leukotrienes mediating essentially every aspect of acute inflammation.

An "inflammatory disorder mediated by LTB_4" or a "LTB_4-mediated disorder", refers to a disorder resulting from an inflammatory response wherein LTB_4 mediation is implicated as a factor by observation of LTB_4 presence at the site of the inflammation.

An "inflammatory disorder of an epithelial tissue" or "of an epithelium" refers to an inflammatory disorder in which one or more epithelial tissues or tissues adjacent to the epithelial layer are affected. Exemplary epithelial tissues

include the epidermal layer of the skin, the cornea epithelium of the eye, and the epithelia associated with the mucosal linings of the respiratory, alimentary, gastrointestinal and urinary tracts.

The term "receptor" refers to a molecular structure or site on the surface or interior of a cell that binds with substances such as hormones, antigens, drugs, or neurotransmitters. An "agonist" at a receptor refers to a drug or other chemical that can bind to a receptor to produce the physiologic reaction that is typical of a naturally occurring substance. An "antagonist" refers to a chemical substance that acts at a receptor to produce a physiologic reaction that is other than the action produced by the natural, endogenous receptor-binding entity or natural ligand. Such antagonist activity may occur when a drug or chemical substance binds the receptor at a much lower concentration than the natural ligand, and thereby displaces the natural ligand and prevents or reduces the amount of receptor binding to the natural ligand.

The term "LTB_4 antagonist" means a chemical substance that competitively binds to the LTB_4 receptor such that (a) the binding of the natural ligand (LTB_4) is inhibited by occupation of the LTB_4 receptor by the LTB_4 antagonist, and (b) the LTB_4 antagonist bound to the LTB_4 receptor does not generate the same physiological response produced by native LTB_4 bound to the LTB_4 receptor.

The term "acyl" means a radical of the general formula –C(=O)-R, wherein –R is hydrogen, hydrocarbyl, amino, alkylamino, dialkylamino hydroxy or alkoxy." Examples include for example, acetyl ($-C(=O)CH_3$), propionyl ($-C(=O)CH_2CH_3$), benzoyl ($-C(=O)C_6H_5$), phenylacetyl ($-C(=O)CH_2C_6H_5$), carboethoxy ($-CO_2CH_2CH_3$), and dimethylcarbamoyl ($-C(=O)N(CH_3)_2$). When the R group in the acetyl radical is alkoxy, alkyl amino or dialkyl amino, the alkyl portion is preferably (C_1-C_6)alkyl, more preferably (C_1-C_3)alkyl. When the R is hydrocarbyl, it is preferably (C_1-C_7)hydrocarbyl. When R is hydrocarbyl, it is preferably alkyl, more preferably (C_1-C_6)alkyl.

The term "alkyl", by itself or as part of another substituent means, unless otherwise stated, a straight, branched or cyclic chain hydrocarbon radical, including di- and multi-radicals, having the number of carbon atoms designated (*i.e.* C_1-C_6 means one to six carbons). Alkyl groups include straight chain, branched chain or cyclic groups, with straight being preferred. Examples include: methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, neopentyl, hexyl, cyclohexyl and cyclopropylmethyl. (C_1-C_6)alkyl is preferred. Most preferred is (C_1-C_3)alkyl, particularly ethyl, methyl and isopropyl.

The term "alkoxy" employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined above, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (isopropoxy) and the higher homologs and isomers. Preferred are (C_1-C_6)alkoxy. More preferred is (C_1-C_3)alkoxy, particularly ethoxy and methoxy.

The term "amine" or "amino" refers to radicals of the general formula -NRR', wherein R and R' are independently selected from hydrogen or a hydrocarbyl radical, or wherein R and R' combined form a heterocycle. Examples of amino groups include: $-NH_2$, methyl amino, diethyl amino, anilino, benzyl amino, piperidinyl, piperazinyl and indolinyl. Preferred hydrocarbyl radicals are (C_1-C_7)hydrocarbyl radicals. Preferred are hydrocarbyl radicals that are alkyl radicals. More preferred are (C_1-C_6)alkyl.

The term "aromatic" refers to a carbocycle or heterocycle having one or more polyunsaturated rings having aromatic character (4n + 2) delocalized π (pi) electrons).

The term "aryl" employed alone or in combination with other terms, means, unless otherwise stated, a carbocyclic aromatic system containing one or more rings (typically one, two or three rings) wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl; anthracyl; and naphthyl.

The term "hydrocarbyl" refers to any moiety comprising only hydrogen and carbon atoms. This definition includes for example alkyl, alkenyl, alkynyl, aryl and benzyl groups. Preferred are (C_1-C_7)hydrocarbyl.

The term "heteroalkyl" by itself or in combination with another term means, unless otherwise stated, a stable straight or branched chain radical consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S. Nitrogen and sulfur atoms may be optionally oxidized to the N-oxide and sulfoxide or sulfone, respectively. In addition, a nitrogen heteroatom may be optionally quaternized. The heteroatom(s) may be placed at any position of the heteroalkyl group, including between the rest of the heteroalkyl group and the fragment to which it is attached, as well as attached to the most distal carbon atom in the heteroalkyl group. Preferred are (C_2-C_6)heteroalkyl. More preferred are (C_2-C_4)heteroalkyl. Examples include: -O-CH_2-CH_2-CH_3, -CH_2-$CH_2$$CH_2$-OH, -$CH_2$-$CH_2$-NH-$CH_3$, -$CH_2$-C(=O)-$CH_3$, -$CH_2$-N=N-$CH_2$-$CH_3$, -$CH_2$-S-$CH_2$-$CH_3$, -$CH_2$$CH_2$-S(=O)-$CH_3$ and –CH_2-CH_2-NH-SO_2-CH_3. Up to two heteroatoms may be consecutive, such as, for example, -CH_2-NH-OCH_3, or –CH_2-CH_2-S-S-CH_3. More preferred are heteroalkyl groups containing one or two oxygen atoms.

When two groups may "combine to form a carbocyclic or heterocyclic 5- or 6-membered ring," a carbocyclic ring is preferably saturated. Preferred heterocyclic rings are saturated rings containing one or two heteroatoms selected from N, O and S. Heterocyclic rings annulated to the benzodiazepine seven-membered ring in this way include, for example, furan, dihydrofuran, tetrahydrofuran, pyran, dihydropyran, tetrahydropyran, thiophene, dihydrothiophene, tetrahydrothiophene, pyrrole, dihydropyrrole, pyrrolidine, pyridine, dihydropyridine, tetrahydropyridine and piperidine.

When two groups may "combine to form a 5-, 6- or 7-membered heterocyclic ring," preferred heterocyclic rings are 5- or 6-membered rings containing one or two heteroatoms selected from N, O and S. More preferred are heterocyclic rings containing one heteroatom selected from N, O and S. Heterocyclic rings annulated to the benzodiazepine phenyl ring in this way

include, for example, furan, dihydrofuran, dioxane, dioxolane, pyran, dihydropyran, tetrahydropyran, thiophene, dihydrothiophene, pyridine, dihydropyridine, tetrahydropyridine, piperidine, pyrrole, dihydropyrrole, imidazole, dihydroimidazole, thiazole, dihydrothiazole, oxazole, and dihydrooxazole.

The term "substituted" means that an atom or group of atoms has replaced hydrogen as the substituent attached to another group. For aryl and heteroaryl groups, the term "substituted" refers to any level of substitution, namely mono-, di-, tri-, tetra-, or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position.

The phrase "optically active" refers to a property whereby a material rotates the plane of plane-polarized light. A compound that is optically active is nonsuperimposable on its mirror image. The property of nonsuperimposablity of an object on its mirror image is called chirality.

The property of "chirality" in a molecule may arise from any structural feature that makes the molecule nonsuperimposable on its mirror image. The most common structural feature producing chirality is an asymmetric carbon atom, *i.e.*, a carbon atom having four nonequivalent groups attached thereto.

The term "enantiomer" refers to each of the two nonsuperimposable isomers of a pure compound that is optically active. Single enantiomers are designated according to the *Cahn-Ingold-Prelog* system, a set of priority rules that rank the four groups attached to an asymmetric carbon. See March, Advanced Organic Chemistry, 4th Ed., (1992), p. 109. Once the priority ranking of the four groups is determined, the molecule is oriented so that the lowest ranking group is pointed away from the viewer. Then, if the descending rank order of the other groups proceeds clockwise, the molecule is designated *R* and if the descending rank of the other groups proceeds counterclockwise, the molecule is designated *S*. In the example below, the *Cahn-Ingold-Prelog* ranking sequence id A > B > C > D. The lowest ranking atom, D is oriented away from the viewer.

R configuration

S configuration

The term "racemate" or the phrase "racemic mixture" refers to a 50-50 mixture of the (*R*)- and (*S*)-enantiomers of a compound such that the mixture does not rotate plane-polarized light.).

The term "substantially isolated", or "substantially free" of the other enantiomer or the term "resolved" or the phrase "substantially free" of the corresponding (*S*)-enantiomer, when used to refer to an optically active compound of formula I, means the (*R*)- and (*S*)-enantiomers of the compound have been separated such that the composition is 80% or more by weight a single enantiomer.

Thus, by "(*R*)-2,3-benzodiazepine substantially free of the (*S*)-enantiomer" is meant a 2,3-benzodiazepine compound that comprises 80% or more by weight of its (*R*)-enantiomer and likewise contains 20% or less of its (*S*)-enantiomer as a contaminant, by weight.

The term "effective amount" when used to describe therapy to an individual suffering from an inflammatory disorder, particularly a LTB_4-mediated inflammatory disorder, refers to the amount of a compound of formula I, or of a combination of a compound of formula I with one or more additional agents, *e.g.*, aminosalicylates, corticosteroids, antimetabolites, immunosuppressants, TNF-α inhibitors, inhibitors of leukotriene synthesis, or leukotriene antagonists, that inhibits the inflammatory process. The inhibition of the inflammatory process results in a therapeutically useful and selective reduction in the symptoms of inflammation when administered to a patient suffering from a disorder which manifests chronic or acute inflammation, particularly inflammation associated with physiologically relevant concentrations of LTB_4. An effective amount of a compound of formula I, or of a combination of a compound of formula I with one or more additional agents, *e.g.*, aminosalicylates, corticosteroids, antimetabolites, immunosuppressants,

TNF-α inhibitors, inhibitors of leukotriene synthesis, or leukotriene antagonists, for the prevention of an inflammatory disorder, particularly a LTB_4-mediated inflammatory disorder, is an amount which prevents or delays the onset of symptoms of an inflammatory disorder in an individual during a time interval coinciding with an increased risk of the inflammatory disorder. The term "individual" or "subject", includes human beings and non-human animals. With respect to the disclosed methods of treating an inflammatory disorders, particularly LTB_4-mediated inflammatory disorders, these terms refer, unless the context indicates otherwise, to an organism that is afflicted with such an inflammatory disorder.

With respect to disclosed methods of preventing inflammatory disorders, particularly, preventing LTB_4-mediated inflammatory disorders, this term refers unless the context indicates otherwise, to an organism that is likely to be afflicted with such an inflammatory disorder. The selection of an individual likely to incur such an inflammatory disorder may take into account the presence of inflammatory conditions that historically are known to have a high incidence of recurrence, such as, for example, IBD. The likelihood of incurring such an inflammatory disorder may also be due to tissue insult that is known beforehand, such as a surgical procedure. The future inflammatory disorder may also result from a secondary effect of an initial tissue insult. An example of this is inflammation due to gout caused by elevated uric acid levels that occur secondary to lysis of a tumor mass following administration of cytotoxic chemotherapy or therapeutic radiation treatment. The term "prevention" in this context also includes a delay in the onset of inflammation or the symptoms thereof or a prolongation of periods of remission in an individual who experiences recurring inflammatory disorders.

Description of the Figures

Fig. 1 is a plot of data gathered in a competitive binding study of the displacement of $[^3H]LTB_4$ by (*R*)-tofisopam from LTB_4 receptors. IC_{50} and K_i values for (*R*)-tofisopam displacement of $[^3H]LTB_4$ were generated.

Fig. 2 is a plot of data gathered in a competitive binding study of the displacement of [^{3}H]LTB$_4$ by racemic tofisopam from LTB$_4$ receptors. IC$_{50}$ and K$_i$ values for racemic tofisopam displacement of [^{3}H]LTB$_4$ were generated.

Fig. 3 is a plot of data gathered in a competitive binding study of the displacement of [^{3}H]LTB$_4$ by (*S*)-tofisopam from LTB$_4$ receptors. IC$_{50}$ and K$_i$ values for (*S*)-tofisopam displacement of [^{3}H]LTB$_4$ were generated.

Detailed Description of the Invention

According to the present invention, 2,3-benzodiazepines of formula I, and pharmaceutically acceptable salts thereof, are antagonists of leukotriene B$_4$. The compounds are useful in methods of treatment or prevention of inflammatory disorders, particularly, inflammatory disorders mediated by leukotriene B$_4$.

Inflammatory disorders believed to be treatable or preventable by methods of the invention include, for example: inflammatory bowel diseases (*e.g.*, CD, UC, indeterminate colitis, and infectious colitis); RA; gout; mucositis (*e.g.*, oral mucositis, gastrointestinal mucositis, nasal mucositis, and proctitis); necrotizing enterocolitis; inflammatory skin disorders (*e.g.*, psoriasis, atopic dermatitis, and contact hypersensitivity); aphthous ulcers; pharyngitis; esophagitis; peptic ulcers; gingivitis; periodontitis; ocular diseases (*e.g.*, conjunctivitis, retinitis, and uveitis); and radiation-induced gastrointestinal inflammation.

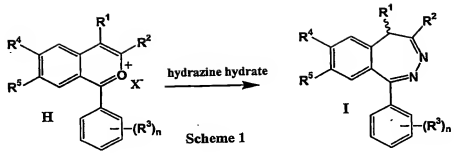
In addition, compounds of formula I are believed useful in the treatment of the above inflammatory disorders in an individual who is also suffering from IBS.

Preparation of 2,3-Benzodiazepines of Formula I.

The (*R*)-2,3-benzodiazepines of formula I useful in the present invention may be prepared by one of several methods. These methods generally follow the synthetic strategies and procedures used in the synthesis of racemic 2,3-benzodiazepines of formula I, such as tofisopam and tofisopam analogs. See U.S. Patent Nos. 3,736,315 and 4,423,044 (tofisopam syntheses) and Horvath *et*

al., Progress in Neurobiology 60(2000) p.309-342 and references cited therein (preparation of tofisopam and analogs thereof), the entire disclosures of which are incorporated herein by reference. In the synthesis methods that follow, the products of the chemical syntheses are racemic mixtures of (R)- and (S)-2,3-benzodiazepines of formula I. These racemic mixtures may be subsequently separated using known methods of resolution to produce the (*R*)-2,3-benzodiazepines of formula I substantially free of the (*S*)-enantiomers. By an "(*R*)-2,3-benzodiazepine" is meant a 2,3-benzodiazepine that has an (*R*) absolute conformation by virtue of a substitution at the 5-position of the benzodiazepine ring to give a resolvable chiral carbon at the 5-position. By an "(*R*)-2,3-benzodiazepine substantially free of the (*S*)-enantiomer" or "an (*R*)-enantiomer of a compound of formula I substantially free of the corresponding (*S*)-enantiomer" is meant a compound that comprises 80% or more by weight of the desired (*R*)-enantiomer and likewise contains 20% or less of the (*S*)-enantiomer as a contaminant, by weight. Preferably, compounds used in methods of the present invention have a composition that is 85% by weight or greater of the (*R*)-enantiomer, and 15% by weight, or less, of the (*S*)-enantiomer. More preferably, compounds used in methods of the present invention have a composition that is 90% by weight or greater of the (*R*)-enantiomer and 10% by weight, or less, of the (*S*)-enantiomer. More preferably, compounds used in methods of the present invention have a composition that is 95% by weight or greater of the (*R*)-enantiomer and 5% by weight, or less, of the (*S*)-enantiomer. Most preferably, compounds used in methods of the present invention have a composition that is 99% by weight or greater of the (*R*)-enantiomer and 1% by weight, or less, of the (*S*)-enantiomer.

Racemic 2,3-benzodiazepines of formula I may be synthesized, as shown in Scheme 1, from the corresponding 2-benzopyrilium salt **H** by reaction with hydrazine hydrate, wherein X^- is a counterion such as, for example perchlorate:



Scheme 1

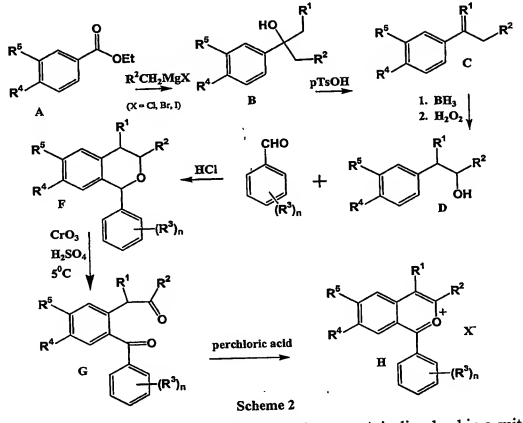
Accordingly, hydrazine hydrate (98%, approximately 3 equivalents based on the 2-benzopyrylium salt) is added dropwise to a stirred solution of the 2-benzopyrylium salt **H** in glacial acetic acid (approximately 1mL/3mmol of 2-benzopyrylium salt). During this operation, the solution is maintained at an elevated temperature, preferably, 80-100°C. The solution is then maintained a higher elevated temperature, preferably 95-100°C, for about one hour. Then the reaction mixture is diluted with 2% aqueous sodium hydroxide solution (approximately 3 equivalents based on the 2-benzopyrylium salt) and cooled. The product 2,3-benzodiazepine separates as a solid and is removed by filtration, washed with water and dried. The crude product may be purified by taking it up in a polar aprotic solvent such as dimethylformamide (DMF) at an elevated temperature, preferably 100-130°C, and decolorizing the solution with activated carbon. The carbon is removed by filtration and the filtered solution is diluted with water. The purified product precipitates out of the solution and is collected by filtration.

See Kórósi *et al.*, US Patent 4,322,346, the entire disclosure of which is incorporated herein by reference, disclosing three variations of the reaction protocol for preparing a substituted 2,3-benzodiazepine from the precursor benzopyrilium salt.

Retrosynthetically, the intermediate benzopyrilium salt, **H**, may be prepared from one of several starting materials. According to one such method, illustrated in Scheme 2, intermediate **H** is prepared from the corresponding aryl ethanol derivative **D** via the isochroman intermediate **F**.

Another variation for preparing 2,3-benzodiazepines is illustrated in Scheme 3 and 4 (Examples 2 and 3). The synthesis there proceeds from intermediate **G** without isolation of the intermediate benzopyrilium salt **H**.

2-Benzopyrylium salts **H** may be synthesized from intermediate 2-substituted phenyl ethanol derivatives **D** through isochroman intermediate **F**, wherein X^- is a counterion such as, for example, perchlorate:



Scheme 2

Accordingly, a substituted benzoic acid ester, **A** is dissolved in a suitable solvent, preferably ether and cooled to 0°C. Two equivalents of a suitable Grignard reagent are added dropwise and the reaction is allowed to warm to room temperature and monitored for disappearance of starting material. When the reaction is complete, it may be quenched with a proton source such as acetic acid. Volatiles are removed *in vacuo*, and the product **B** is used for the next step without purification.

The α,α-substituted benzyl alcohol **B** is taken up in a high boiling solvent such as toluene and a catalytic amount of para-toluene sulfonic acid (p-TsOH). The mixture is warmed to reflux and may be monitored for disappearance of starting materials. When the reaction is complete, the volatiles are removed in vacuo and the crude product **C** is purified by column chromatography.

The substituted styrene **C** is hydroxylated under anti-Markovnikov conditions to give intermediate phenylethyl alcohol **D**. A solution of **D**, and of a suitably substituted benzaldehyde **E** (1.2 eq) are added to anhydrous dioxane. The resulting solution is then saturated with gaseous HCl and warmed, preferably to reflux temperature for about one hour. The mixture is then cooled to room temperature, poured into water, basified (preferably with aqueous sodium hydroxide) and extracted with an organic solvent (preferably ethyl acetate). The extract is dried, filtered and concentrated under vacuum. The resulting residue is purified, preferably by crystallization, to yield **F**.

To a stirred, cooled, (preferably to 0-5°C) solution of **F** (2g) in acetone (30mL), is added dropwise a solution of chromium trioxide (2g) in 35% sulfuric acid (20mL). The latter solution is added at a rate such that the reaction temperature remains below 5° C. After the addition is complete, the reaction mixture is allowed to rise to room temperature and is stirred at room temperature for two hours. The reaction mixture is then poured into water and extracted with an organic solvent, preferably ethyl acetate. The organic extract is washed with water and then with ice-cold 10% aqueous sodium hydroxide. The aqueous alkaline fraction is then acidified, preferably with dilute aqueous hydrochloric acid, and extracted with an organic solvent, preferably, chloroform. The chloroform extract is dried, filtered and concentrated under vacuum to give **G**. The crude residue may further be purified by column chromatography.

The 2-α-acyl hydrocarbylbenzophenone **G** (5g) is dissolved in glacial acetic acid (15 mL). To this mixture is added 60% perchloric acid (7.5 mL). The resulting mixture is warmed to 100°C (steam bath) for three minutes. The mixture is allowed to cool to room temperature. Crystallization of the crude product may begin spontaneously at this point or may be induced by addition of ether or ethyl acetate. The product 2-benzopyrylium salt **H** is removed by filtration and purified by recrystallization, preferably from ethanol or glacial acetic acid/ethyl acetate.

A similar synthetic sequence for preparation of 2,3-benzodiazepines is disclosed in US Patent 3,736,315, the entire disclosure of which is incorporated herein by reference. Synthetic strategies for preparation of 2,3-benzodiazepines are also disclosed in Horvath *et al., Progress in Neurobiology* 60(2000) p309-342 and references cited therein; the entire disclosures of which are incorporated herein by reference.

Alternative methods for preparation of intermediate **H** start with an aryl acetonide or indanone starting material. See Kunnetsov, E.V., and Dorofeenko, G.N., *Zh. Org. Khim.*, 6, 578-581. and M. Vajda, *Acta Chem. Acad. Sci. Hung.*, 40, p.295-307, 1964, respectively, the entire disclosures of which are incorporated herein by reference.

To synthesize a 2,3-benzodiazepine derivative of formula I having an amine substituent, the starting aromatic amine components must be protected with a protecting group or otherwise rendered unreactive in order for the amine to be rendered stable to the reaction conditions employed in the reaction schemes shown or referenced above. A means of circumventing the need for a protecting group may be to use a starting material containing an aromatic nitro group(s) in place of the desired aromatic amino group(s). The nitro group performs the same function as an amine protecting group in this synthesis and it may, following the synthesis steps that are incompatible with an amine substituent, be then reduced to an amine. Reduction of the aromatic nitro group can be done, for example, via catalytic hydrogenation. Catalytic hydrogenation provides the capability to selectively reduce the aromatic nitro group without reducing the olefin or other functionality in the intermediate. This synthetic strategy is disclosed in US Patent 4,614,740, wherein racemic 2,3-benzodiazepines were prepared with amino groups at a position corresponding the R^3 of formula I of the present invention. The entire disclosure of US 4,614,740 is incorporated herein by reference.

Resolution of (*R*)-2,3-Benzodiazepines of Formula I.

The synthetic procedures shown (or referenced) above produce racemic mixtures of 2,3-benzodiazepines of formula I that are useful in methods of the

present invention. In order to prepare the preferred (*R*)-2,3-benzodiazepines of formula I that are useful in methods of the present invention, the racemic mixture must be resolved.

Racemic 2,3-benzodiazepines of formula I may, for example, be converted to the (*S*)-dibenzoyltartaric acid salt, which is a diastereomeric mixture of SS and RS configurations. The pair of diastereomers (*R,S*) and (*S,S*) possess different properties, *e.g.*, differential solubilities, that allow for the use of conventional separation methods. Fractional crystallization of diastereomeric salts from a suitable solvent is one such separation method. This resolution has been successfully applied to the resolution of racemic tofisopam. See Hungarian Patent 178516 and also Toth *et al., J. Heterocyclic. Chem.*, 20:09-713 (1983), the entire disclosures of which are incorporated herein by reference.

Alternatively, racemic-2,3-benzodiazepines of formula I may be derivatized via, for example, acylation of an aryl hydroxy moiety, with a chiral acylating reagent, *e.g.*, (*S*)-mandelic acid. The resulting ester, has a second chiral center, and thus exists as a diastereomeric pair separable using conventional methods such as crystallization or chromatography. Following the separation, the chiral moiety with which the racemic 2,3-benzodiazepine is derivatized, may be removed.

Racemic 2,3-benzodiazepines of formula I may be separated without diastereomer formation by differential absorption on a chiral stationary phase of a chromatography column, particularly a preparative HPLC column. Chiral HPLC columns are commercially available with a variety of packing materials to suit a broad range of separation applications. Exemplary stationary phases suitable for resolving the racemic 2,3-benzodiazepines of formula I include:

(i) macrocyclic glycopeptides, such as silica-bonded vancomycin which contains 18 chiral centers surrounding three pockets or cavities;

(ii) chiral α_1-acid glycoprotein;

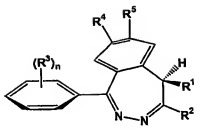
(iii) human serum albumin; and

(iv) cellobiohydrolase (CBH).

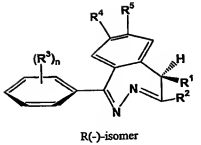
Chiral α_1-acid glycoprotein is a highly stable protein immobilized onto spherical silica particles that tolerates high concentrations of organic solvents, high and low pH, and high temperatures. Human serum albumin, though especially suited for the resolution of weak and strong acids, zwitterionic and nonprotolytic compounds, has been used to resolve basic compounds. CBH is a very stable enzyme which has been immobilized onto spherical silica particles and is preferentially used for the separation of enantiomers of basic drugs from many compound classes.

The resolution of tofisopam by chiral chromatography using macrocyclic glycopeptide as a stationary phase on a Chirobiotic V™ column (ASTEAC, Whippany, NJ) is disclosed in US Patent 6,080,736. Fitos *et al.* (*J. Chromatogr.*, 709 265 (1995)), discloses another method for resolving racemic tofisopam by chiral chromatography using a chiral α_1-acid glycoprotein as a stationary phase on a CHIRAL-AGP™ column (ChromTech, Cheshire, UK). The latter method separates the (*R*)- and (*S*)- enantiomers and also resolves the two conformers (discussed below) of each enantiomer. These chromatographic methods, may be used generally to separate racemic 2,3-benzodiazepines of formula I into individual (*R*)- and (*S*)-enantiomers. The Chirobiotic V™ column is available in a semi-preparative size as employed for the above separation 500mm x 10mm). The stationary phase of the Chirobiotic V™ column is commercially available in bulk for packing of preparative chromatography columns with larger sample capacity.

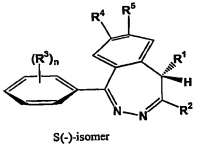
(*R*)- and (*S*)-enantiomers of 2,3-benzodiazepines may also exist in two stable conformations that may be assumed by the benzodiazepine ring, as generally depicted below:



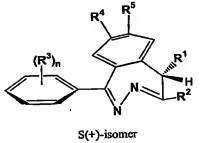
R(+)-isomer



R(-)-isomer



S(-)-isomer



S(+)-isomer

The present invention includes methods as described herein that use any and all observable conformations of compounds of formula I, preferably compounds having the (*R*)-absolute configuration at carbon 5 of the benzodiazepine ring, which are biologically active in treatment or prevention of inflammatory disorders, particularly inflammatory disorders mediated by LTB_4..

It will be understood that compounds of formula I useful in the methods of the present invention may contain one or more chiral centers in addition to chiral center at the 5-position of the benzodiazepine ring of compounds of formula I. Such compounds may exist in, and may be isolated as pure enantiomeric or diastereomeric forms or as racemic mixtures. The present invention therefore includes methods that use any possible enantiomers, diastereomers, racemates or mixtures thereof of formula I (dictated by a chiral center other than the 5-position of the benzodiazepine ring) which are biologically active in the treatment or prevention of inflammatory disease states, particularly inflammatory disease states mediated by LTB_4.

Salts of Compounds of Formula I.

The compounds used in the methods of the present invention may take the form of pharmaceutically-acceptable salts. The term "salts", embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The term "pharmaceutically-acceptable salt" refers to salts that

possess toxicity profiles within a range so as to have utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in a synthetic process or in the process of resolving enantiomers from a racemic mixture. Suitable pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, example of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, salicyclic, salicyclic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, algenic, beta-hydroxybutyric, salicyclic, galactaric and galacturonic acid.

Suitable pharmaceutically acceptable base addition salts of compounds of formula I useful in methods of the invention include for example, metallic salts made from calcium, magnesium, potassium, sodium and zinc or organic salts made from *N,N'*-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (*N*-methylglucamine) and procaine. All of these salts may be prepared by conventional means from the corresponding compound of formula I by reacting, for example, the appropriate acid or base with the compound of formula I.

Administration of Compounds of Formula I.

The compounds useful in methods of the invention may be administered to individuals (mammals, including animals and humans) afflicted with inflammatory disorders, particularly individuals afflicted with LTB_4-mediated inflammatory disorders.

For prophylactic administration, the compounds useful in the practice of methods of the invention should be administered far enough in advance of a known event that increases the chance of an inflammatory disorder, particularly an inflammatory disorder mediated by LTB_4, such that the compound is able to reach the site of action in sufficient concentration to exert an effect. The pharmacokinetics of specific compounds may be determined by means known in the art and tissue levels of a compound in a particular individual may be determined by conventional analyses.

One or more compounds useful in the practice of the present inventions may be administered simultaneously, or different compounds useful in the practice of the present invention may be administered at different times during treatment or prevention therapy.

In addition, compounds of formula I may be administered for treatment of inflammatory disorders, in combination with one or more additional therapeutic agents. Such additional agents include aminosalicylates, corticosteroids, antimetabolites, immunosuppressants, TNF-α inhibitors, inhibitors of leukotriene synthesis, and leukotriene antagonists; wherein when the additional therapeutic agents are leukotriene antagonist, they are other than compounds of formula I.

Aminosalicylates believed useful in combination with compounds of formula I in methods of the invention include, for example, sulfasalazine and mesalamine.

Corticosteroids believed useful in combination with compounds of formula I in methods of the invention include, for example, prednisone and budesonide.

Antimetabolites believed useful in combination with compounds of formula I in methods of the invention include, for example, azathioprine.

Immunosuppressants believed useful in combination with compounds of formula I in methods of the invention include, for example, cyclosporine and tacrolimus.

TNF-α inhibitors believed useful in combination with compounds of formula I in methods of the invention include, for example, infliximab, etanercept, and adalimumab.

Inhibitors of leukotriene synthesis believed useful in combination with compounds of formula I in methods of the invention include, for example, 5-LO inhibitors, *e.g.*, ETH615, linetastine, lonapalene (RS43179), MK 886, and zileuton. Other inhibitors of leukotriene synthesis believed useful in combination with compounds of formula I include, for example, 15-HETE and leflunomide.

Leukotriene antagonists useful in combination with compounds of formula I in methods of the invention include, for example, SC41930, SC53228, CGS-25019C, ONO-4057, SB-202247, VML295 (LY293111), CP-105696, CP-195543, and U-75302.

Routes of Administration.

The compounds useful in methods of the invention may be administered for therapeutic effect by any route, for example enteral (*e.g.*, oral, rectal, intranasal, *etc.*) and parenteral administration. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intravaginal, intravesical (*e.g.*, into the bladder), intradermal, topical or subcutaneous administration. Also contemplated within the scope of the invention is the instillation of drug in the body of the patient in a controlled formulation, with systemic or local release of the drug to occur at a later time. For anti-inflammatory use, the drug may be localized in a depot for controlled release to the circulation, or controlled release to a local site of inflammation.

As discussed, the invention can be used, for instance, to prevent or treat an inflammatory skin disorder such as psoriasis, atopic dermatitis, and contact hypersensitivity. Other indications of interest include dry skin (sometimes called "winter itch"). In these invention examples, topical application of at least one of the compounds of Formula I (e.g., one, two or three) will be often be indicated for some indications.

Topical formulations in accord with the invention typically include a topical vehicle suitable for administration to the subject (particularly a mammal such as a human patient) in an amount suitable to reduce, inhibit or eliminate existing or potential skin irritation or inflammation. More specific formulations for topical use preferably less than about 100mg/ml of one or more of the compounds provided above as Formula I, more preferably between from about 0.001mg/ml to about 50 mg/ml, even more preferably between from about 0.01 mg/ml to about 10 mg/ml with about 0.1 mg/ml to about 5 mg/ml being suitable for many applications. Thus, the administered dose may be less than about 100 mg/day, more preferably less than about 50 mg/day, even more preferably less than about 10 mg or 5 mg/day and yet more preferably less than about 1 mg/day.

Optimal topical concentrations of one or more of the compounds of Formula I can sometimes be adjusted (generally decreased) if one or more other anti-inflammatory component is included in the formulation. In particular, it is contemplated that lower (eg., one-half less) amounts of compound may be used, while still maintaining comparable levels of anti-inflammation activity on the skin, by further including an approximately equal concentration of, for example a steroid or non-steroidal anti-inflammatory agent.

In embodiments in which it is desirable to topically administer a salt of one or more of the compounds according to Formula I, a variety of suitable salts can be employed such as those mentioned above. Appropriate formulations generally suited for topical use include such vehicles (or vehicle components) as water; organic solvents such as alcohols (particularly lower alcohols readily capable of evaporating from the skin such as ethanol), glycols (such as glycerin), aliphatic alcohols (such as lanolin); mixtures of water and organic solvents (such as water and alcohol), and mixtures of organic solvents such as alcohol and glycerin (optionally also with water); lipid-based materials such as fatty acids, acylglycerols (including oils, such as mineral oil, and fats of natural or synthetic origin), phosphoglycerides, sphingolipids and waxes; protein-based materials such as collagen and gelatin; silicone-based materials (both non-

volatile and volatile) such as cyclomethicone, demethiconol and dimethicone copolyol (Dow Corning); hydrocarbon-based materials such as petrolatum and squalane; anionic, cationic and amphoteric surfactants and soaps; sustained-release vehicles such as microsponges and polymer matrices; stabilizing and suspending agents; emulsifying agents; and other vehicles and vehicle components that are suitable for administration to the skin, as well as mixtures of topical vehicle components as identified above or otherwise known to the art. The vehicle may further include components adapted to improve the stability or effectiveness of the applied formulation, such as preservatives, antioxidants, skin penetration enhancers, sustained release materials, and the like. Examples of such vehicles and vehicle components are well known in the art and are described in such reference works as Martindale--The Extra Pharmacopoeia (Pharmaceutical Press, London 1993) and Martin (ed.), Remington's Pharmaceutical Sciences.

The choice of a suitable vehicle will depend on the particular physical form and mode of delivery that the formulation is to achieve. Examples of suitable forms include liquids (including dissolved forms of the cations of the invention as well as suspensions, emulsions and the like); solids and semisolids such as gels, foams, pastes, creams, ointments, "sticks" (as in lipsticks or underarm deodorant sticks), powders and the like; formulations containing liposomes or other delivery vesicles; rectal or vaginal suppositories, creams, foams, gels or ointments; and other forms. Typical modes of delivery for use as an anti-inflammatory for the skin include application using the fingers; application using a physical applicator such as a cloth, tissue, swab, stick or brush (as achieved for example by soaking the applicator with the formulation just prior to application, or by applying or adhering a prepared applicator already containing the formulation--such as a treated or premoistened bandage or patch, wipe, washcloth or stick--to the skin); spraying (including mist, aerosol or foam spraying); dropper application (as for example with ear or eye drops); sprinkling (as with a suitable powder form of the formulation); and soaking.

Other "soothing" ingredients for optional use with the topical applications of the invention include, but are not limited to glycerin, aloe vera, chamomile, cola nitida extract, green tea extract, tea tree oil, licorice extract, allantoin, urea, caffeine or other xanthines, and glycyrrhizic acid and its derivatives may also be beneficially used with the invention to help reduce or block unwanted inflammation of the skin.

As also discussed, it is an object of the present invention to address one or a combination of gastrointestinal disorders in a mammal such as a human patient by administering an effective amount the compound of Formula I. A variety of particular administration routes can be employed such as those known to target the alimentary canal, stomach, small intestine or colon. Of particular interest is the delivery of the compounds intracolonically, for example by suppository or enema.

See, for instance, Groning R., et al., *Drug Dev. Ind Pharm*, ID:527-39 (1984); Sheth, P. R., *Drug Dev. Ind. Pharm.* 10:313-39 (1983); Chien, Y. W., *Drug Dev. Ind. Pharm* 9:1291-330 (1983); Desai, S. and Bolton, S., *Pharm. Res.* 10: 1321-5 (1993)); Banakar (Amer. Pharm. 27: 39-48 (1987); (Cargill, R., et al., Pharm. Res 5:533-536 (1988); Cargill, R., et al., Pharm. Res. 5:506-509 (1989); (Ritschel, W. A., Angewante Biopharmazie, Stuttgart (1973), pp. 396-402; Agyilirah, G. A., et al., *Polymers for Enteric Coating Applications* in Polymers for Controlled Drug Delivery, Tarcha, P. J. ed., CRC Press, (1991) Boca Raton, pp. 39-66); Magersohn, M., *Modern Pharmaceutics*, Marcel Dekker, New York (1979), pp. 23-85); Ritschel, W. A., *Meth Find Ex. Clin. Pharmacol* 13(5):313-336 (1991); Ritschel, W. A. Angewndte Biopharmazio, Stuttgart Wissensec. Verlag (1973), pp 396-402; Agyilirah, G. A. and Banker, G. S., Polymers for Enteric Coating Applications, *ibid*, pp.39-66).

See also U.S. Pat. Nos. 5,525,634; 4,627,850; 4,904,474; 6,531,152; WO97/25979 (disclosing other gastrointestinal tract application routes).

In one invention embodiment, any of the delivery devices disclosed in U.S. Pat. No. 6,531,152 can be used to localize release of a desired agent in the gastrointestinal tract of the animal. Preferably, such a device includes a core

that includes at least one of the compounds of Formula I. The compounds can be used as the sole active agent or as already discussed can be combined with one or more other agents to address the gastrointestinal indication. In this embodiment, nearly any suitable amount of the compound can be used. However, for many uses, the core diameter can range from 1 mm to 15 mm with a coating level ranging from 2 to 50 mg/cm^2, for instance.

In general, formulations for targeting a compound of Formula I to the gastrointestinal tract typically contain preferably less than about 100mg/ml of one or more of the compounds provided above as Formula I, more preferably between from about 0.001mg/ml to about 50 mg/ml, even more preferably between from about 0.01 mg/ml to about 10 mg/ml with about 0.1 mg/ml to about 5 mg/ml being suitable for many applications. Thus, the administered dose may be less than 100 mg/day, more preferably less 50 mg/day, even more preferably less than 10 mg or 5 mg/day, and yet more preferably less than 1 mg/day of a compound of Formula I.

Pharmaceutical Compositions.

The methods of the present invention may comprise administering 2,3-benzodiazepines, preferably (*R*)-2,3-benzodiazepines, in the form of a pharmaceutical composition, in combination with a pharmaceutically acceptable carrier. The active ingredient in such formulations may comprise from 0.1 to 99.99 weight percent. By "pharmaceutically acceptable carrier" is meant any carrier, diluent or excipient which is compatible with the other ingredients of the formulation and to deleterious to the recipient.

The active agent is preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice. The active agent may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See Alphonso Gennaro, ed., *Remington's Pharmaceutical Sciences,* 18th Ed., (1990) Mack Publishing Co., Easton, PA. Suitable dosage forms may comprise, for example, tablets, capsules, solutions, parenteral solutions, troches, suppositories, or suspensions.

For parenteral administration, the active agent may be mixed with a suitable carrier or diluent such as water, an oil (particularly a vegetable oil), ethanol, saline solution, aqueous dextrose (glucose) and related sugar solutions, glycerol, or a glycol such as propylene glycol or polyethylene glycol. Solutions for parenteral administration preferably contain a water-soluble salt of the active agent. Stabilizing agents, antioxidizing agents and preservatives may also be added. Suitable antioxidizing agents include sulfite, ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol. The composition for parenteral administration may take the form of an aqueous or nonaqueous solution, dispersion, suspension or emulsion.

For oral administration, the active agent may be combined with one or more solid inactive ingredients for the preparation of tablets, capsules, pills, powders, granules or other suitable oral dosage forms. For example, the active agent may be combined with at least one excipient such as fillers, binders, humectants, disintegrating agents, solution retarders, absorption accelerators, wetting agents absorbents or lubricating agents. According to one tablet embodiment, the active agent may be combined with carboxymethylcellulose calcium, magnesium stearate, mannitol and starch, and then formed into tablets by conventional tableting methods.

The compositions of the present invention can also be formulated so as to provide slow or controlled-release of the active ingredient therein. In general, a controlled-release preparation is a composition capable of releasing the active ingredient at the required rate to maintain constant pharmacological activity for a desirable period of time. Such dosage forms can provide a supply of a drug to the body during a predetermined period of time and thus maintain drug levels in the therapeutic range for longer periods of time than other non-controlled formulations.

For example, U.S. Patent No. 5,674,533 discloses controlled-release compositions in liquid dosage forms for the administration of moguisteine, a potent peripheral antitussive. U.S. Patent No. 5,059,595 describes the

controlled-release of active agents by the use of a gastro-resistant tablet for the therapy of organic mental disturbances. U.S. Patent No. 5, 591,767 discloses a liquid reservoir transdermal patch for the controlled administration of ketorolac, a non-steroidal anti-inflammatory agent with potent analgesic properties. U.S. Patent No. 5,120,548 discloses a controlled-release drug delivery device comprised of swellable polymers. U.S. Patent No. 5,073,543 discloses controlled-release formulations containing a trophic factor entrapped by a ganglioside-liposome vehicle. U.S. Patent No. 5,639,476 discloses a stable solid controlled-release formulation having a coating derived from an aqueous dispersion of a hydrophobic acrylic polymer. U.S. Patent No. 6,531,152 discloses, for instance, an immediate release gastrointestinal drug delivery system in which release of drugs in the gastrointestinal tract can be controlled in a location- and time-dependent manner. The patents cited above are incorporated herein by reference in their entirety.

Biodegradable microparticles can be used in the controlled-release formulations of this invention. For example, U.S. Patent No. 5,354,566 discloses a controlled-release powder that contains the active ingredient. U.S. Patent No. 5,733,566 describes the use of polymeric microparticles that release antiparasitic compositions. These patents are incorporated herein by reference.

The controlled-release of the active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. Various mechanisms of drug release exist. For example, in one embodiment, the controlled-release component can swell and form porous openings large enough to release the active ingredient after administration to a patient. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, such as polymers, polymer matrices, gels, permeable membranes, liposomes and/or microspheres, that facilitate the controlled-release of the active ingredient (*e.g.*, (*R*)-tofisopam or a pharmaceutically-acceptable salt thereof) in the pharmaceutical composition. In another embodiment, the controlled-release component is biodegradable, induced by exposure to the aqueous environment,

pH, temperature, or enzymes in the body. In another embodiment, sol-gels can be used, wherein the active ingredient is incorporated into a sol-gel matrix that is a solid at room temperature. This matrix is implanted into a patient, preferably a mammal, having a body temperature high enough to induce gel formation of the sol-gel matrix, thereby releasing the active ingredient into the patient.

The practice of the invention is illustrated by the following non-limiting examples.

Examples

Example 1: Synthesis of 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine.

4.41 g (10mmol) of 1-(3,4-dimethoxyphenyl)-3-methyl-4-ethyl-6,7-dimethoxyisobenzopyrilium chloride hydrochloride is dissolved in methanol (35mL) at a temperature of 40°C. After cooling to 20-25°C, hydrazine hydrate (0.75g, 15mmol, dissolved in 5mL methanol) is added. The reaction is monitored by HPLC and when complete, is evaporated to dryness. The residue is triturated with cold water (3mL), filtered and dried to yield the crude (*R,S*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine which is subsequently triturated with hot ethyl acetate to yield the pure product.

Example 2: Resolution of 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine to yield (*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine.

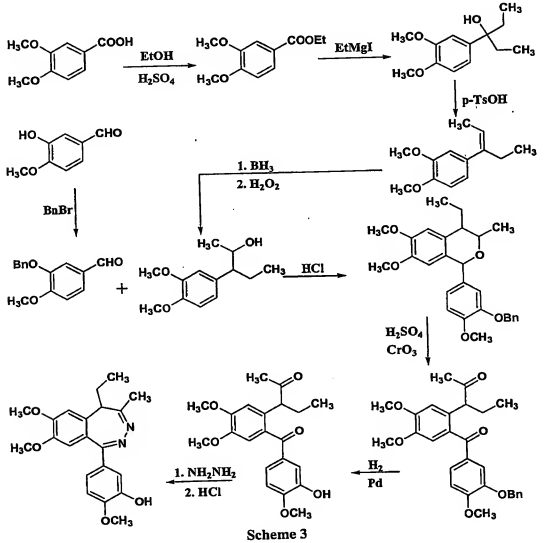
(*R,S*)-1-(3,4-Dimethoxyphenyl)-4-methyl-5-ethyl-7-hydroxy-8-methoxy-5H-2,3-benzodiazepine (43mg, dissolved in acetonitrile) is injected onto a Chirobiotic V column (ASTEAC, Whippany, NJ) Elution of the racemate with methyl-*tert*-butyl ether/acetonitrile 90/10 (v/v), at 40mL/minute, is monitored at 310nm, 2mm path.

The *R*(+) enantiomer is the first compound to elute, and is collected and dried. The *R*(-), *S*(+), *S*(-) enantiomers, and some residual *R*(+) enantiomer

coelute and are collected in subsequent fractions. Approximately 20% of the *R*(+) isomer is converted to the *R*(-) isomer if left in the eluent for 24 hours. A stable 80/20 equilibrium (*R*(+) to *R*(-)) is observed between the conformers in the eluent solution.

Example 3: Synthesis of racemic-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine

Racemic-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine was synthesized according to the route of Scheme 3.



Scheme 3

A. Esterification of 3,4-dimethoxybenzoic acid to yield ethyl-3,4-dimethoxybenzoate([3943-77-9]).

A solution of 200g of 3,4-dimethoxybenzoic acid and 35g of concentrated sulfuric acid in 600mL of absolute ethanol was heated at reflux overnight. The mixture was concentrated and the residue poured into water. Methylene chloride was added and the solution washed successively with water, dilute sodium bicarbonate and water, then dried and concentrated. The residue was recrystallized from acetone/hexane.

B. Addition of ethyl magnesium iodide to ethyl-3,4-dimethoxybenzoate acid to yield 3-(3,4-dimethoxyphenyl)pentan-3-ol.

A solution of 4.8mL of iodoethane in 20mL of ether was added dropwise to a suspension of 1.5g of magnesium turnings in 10mL of ether. After 5mL of the iodoethane solution had been added, a few grains of iodine were added and the mixture was heated to induce formation of the Grignard reagent. The remaining iodoethane solution was then added. After the Grignard formation was complete, a solution of 5g of ethyl 3,4-dimethoxybenzoate in ether was added and the mixture was allowed to stir at room temperature overnight. The reaction was quenched by addition of saturated ammonium chloride. The mixture was extracted with ether. The combined ether extracts were dried and concentrated to an oily residue. Yield: 5g.

C. Elimination of H_2O from 3-(3,4-dimethoxyphenyl)pentan-3-ol to yield 4-((1Z)-1-ethylprop-1-enyl)-1,2-dimethoxybenzene.

A solution of 5g of crude 3-(3,4-dimethoxyphenyl)pentan-3-ol and 0.25g of p-tolenesulfonic acid in 80mL of benzene was heated at reflux for 1hr with azeotropic removal of water. The mixture was then filtered through a pad of sodium bicarbonate and the filtrate concentrated. The residue was purified by distillation under reduced pressure. Yield: 2.9g.

D. Addition of H_2O to 4-((1Z)-1-ethylprop-1-enyl)-1,2-dimethoxybenzene to yield 3-(3,4-dimethoxyphenyl)pentan-2-ol.

To a solution of 26g of 4-((1Z)-1-ethylprop-1-enyl)-1,2-dimethoxybenzene in tetrahydrofuran (THF) at 0°C was added 189mL of a 1.0M solution of borane-THF complex in THF. The mixture was stirred for 3hr at 0°C, then 35.6mL of 50% hydrogen peroxide was added, with simultaneous

addition of 5M sodium hydroxide to maintain the mixture at pH 8. The mixture was extracted with ether. The combined ether extracts were dried and concentrated.

E. Benzylation of 3-hydroxy-4-methoxybenzaldehyde to yield 4-methoxy-3-(phenylmethoxy)benzaldehyde ([6346-05-0]).

A solution of 100g of 3-hydroxy-4-methoxybenzaldehyde and 135g of benzyl bromide in 500mL of acetone containing a suspension of 137g of potassium carbonate was heated at reflux overnight. The mixture was filtered, the filtrate concentrated and the residue recrystallized from toluene/hexane. Yield: 65g.

F. Reaction of 3-(3,4-dimethoxyphenyl)pentan-2-ol with 4-methoxy-3-(phenylmethoxy)benzaldehyde to yield 4-(4-ethyl-6,7-dimethoxy-3-methyliso-chromanyl)-1-methoxy-2-(phenylmethoxy)benzene.

A solution of 14g of 4-methoxy-3-(phenylmethoxy)benzaldehyde and 15g of 3-(3,4-dimethoxyphenyl)pentan-2-ol in 0.3L of dioxane was saturated with hydrogen chloride gas. The mixture was heated at reflux for 3hr, saturated again with hydrogen chloride gas and allowed to stir at room temperature overnight. It was then poured into water, basified with dilute sodium hydroxide and extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated.

G. Ring-opening of 4-(4-ethyl-6,7-dimethoxy-3-methyliso-chromanyl)-1-methoxy-2-(phenylmethoxy)benzene to yield 3-(4,5-dimethoxy-2-{[4-methoxy-3-(phenylmethoxy)phenyl]carbonyl}phenyl)pentan-2-one.

To a solution of 30g of crude 4-(4-ethyl-6,7-dimethoxy-3-methyliso-chromanyl)-1-methoxy-2-(phenylmethoxy)benzene in 450mL of acetone at 5°C was added a solution of 30g of chromic oxide in 300mL of 35% sulfuric acid. The mixture was stirred at room temperature for 2hr, neutralized by adding cold 10% sodium hydroxide and concentrated to remove acetone. Then, water was added and the mixture was extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated. The residue was purified by column chromatography on silica gel. Yield: 10g.

H. Debenzylation of 3-(4,5-dimethoxy-2-{[4-methoxy-3-(phenylmethoxy)-phenyl]carbonyl}phenyl)pentan-2-one to yield 3-{2-[(3-hydroxy-4-methoxy-phenyl)carbonyl]-4,5-dimethoxyphenyl}pentan-2-one.

A solution of 10g of 3-(4,5-dimethoxy-2-{[4-methoxy-3-(phenylmethoxy)-phenyl]carbonyl}phenyl)pentan-2-one in methylene chloride containing a suspension of 0.9g of 10% palladium on carbon was hydrogenated at 80psi for 1hr. The mixture was filtered through diatomaceous earth and the filtrate concentrated. Yield: 6.5g.

I. Annulation of 3-{2-[(3-hydroxy-4-methoxyphenyl)carbonyl]-4,5-dimethoxyphenyl}pentan-2-one by reaction with hydrazine to yield 1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine.

A solution of 6.5g of 3-{2-[(3-hydroxy-4-methoxyphenyl)carbonyl]-4,5-dimethoxyphenyl}pentan-2-one and 2.2mL of hydrazine in 130mL of ethanol was heated at reflux for 0.5hr. After allowing the solution to cool to room temperature, it was saturated with HCl gas. The mixture was then concentrated to a volume of about 5mL, basified with concentrated ammonium hydroxide, and extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated, and the residue recrystallized from ethyl acetate/hexane. Yield: 0.97g

The product 1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine was analyzed by HPLC, elemental analysis, GC/MS, proton NMR and differential scanning calorimetry (DSC). The data are as follows:

Purity: 99.29% by HPLC (% area). Column: Betasil Phenyl 4.6 x 150mm. Mobile Phase: Acetonitrile::0.01M Phosphate Buffer (70::30). Flow Rate: 0.5mL/min. Wavelength: 254nm.

GC-MS; M/e = 358; with the fragmentation pattern matching the proposed structure.

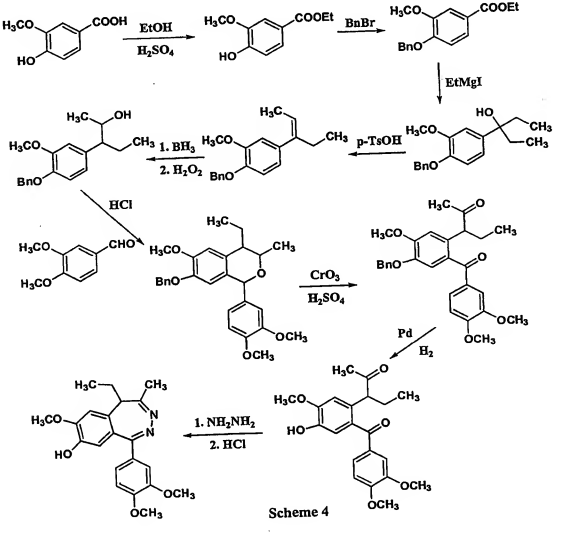
DSC: Temperature program 100°C to 300°C at 5°C/min, indicated molar purity = 99.75% and melting point of 158.6°C.

Elemental analysis (calculated/analysis): %C - 68.09/68.08; %H - 6.61/6.57; N - 7.53/7.35. Calculated values include 0.02 equivalents of ethyl acetate and 0.09 equivalents of residual water.

NMR ($DCCl_3$) (performed on GE QE 300): 1.08ppm (t, 3H); 1.99 (s, 3H); 2.11 (m, 2H); 2.75 (m, 1H); 3.75 (s, 3H); 3.93 (s, 3H); 3.97 (s, 3H); 6.46 (bs, 1H); 6.72 (s, 1H); 6.86 (m, 2H); 7.18 (d, 1H); 7.48 (s, 1H).

Example 4: Synthesis of 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine

Racemic 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine was synthesized according to the route of Scheme 4.



Scheme 4

A. Esterification of 3-methoxy-4-hydroxybenzoic acid to yield ethyl-3-methoxy-4-hydroxybenzoate.

A solution of 100g of 3-methoxy-4-hydroxybenzoic acid and 17g of concentrated sulfuric acid in 300mL of absolute ethanol was heated at reflux overnight. The mixture was concentrated and the residue poured into water. Methylene chloride was added and the solution washed successively with water, dilute sodium bicarbonate and water, then dried and concentrated. Yield: 118g

B. Benzylation of ethyl-3-methoxy-4-hydroxybenzoate to yield ethyl-3-methoxy-4-benzyloxybenzoate.

A solution of 118g of ethyl-3-methoxy-4-hydroxybenzoate and 86mL of benzyl bromide in 600mL of acetone containing a suspension of 124g of potassium carbonate was heated at reflux overnight. The mixture was filtered, the filtrate concentrated and the residue recrystallized from acetone.

C. Addition of ethyl magnesium iodide to ethyl-3-methoxy-4-benzyloxybenzoate to yield 3-(3-methoxy-4-benzyloxyphenyl)pentan-3-ol.

Iodoethane (112mL) was added dropwise to a suspension of 35g of magnesium turnings in 160mL of ether. After the formation of ethyl magnesium iodide was complete, a solution of 142g of ethyl 3-methoxy-4-benzyloxybenzoate in ether was added and the mixture was allowed to stir at room temperature for 3 days. The reaction was quenched by addition of saturated ammonium chloride. The layers were separated and the ether layer was dried and concentrated to an oily residue. Yield: 110g.

D. Elimination of H_2O from 3-(3-methoxy-4-benzyloxyphenyl)pentan-3-ol to yield 4-((1Z)-1-ethylprop-1-enyl)-1-benzyloxy-2-methoxybenzene.

A solution of 110g of crude 3-(3-methoxy-4-benzyloxyphenyl)pentan-3-ol and 7g of p-tolenesulfonic acid in 2L of benzene was heated at reflux for 4hr with azeotropic removal of water. The mixture was then filtered through a pad of sodium bicarbonate and the filtrate concentrated. The residue was purified by column chromatography on neutral alumina.

E. Addition of H_2O to 4-((1Z)-1-ethylprop-1-enyl)-1-benzyloxy-2-methoxybenzene to yield 3-(3-methoxy-4-benzyloxyphenyl)pentan-2-ol.

To a solution of 96g of 4-((1Z)-1-ethylprop-1-enyl)-1-benzyloxy-2-methoxybenzene in THF at 0°C was added 510mL of a 1.0M solution of borane-THF complex in THF. The mixture was stirred for 3hr at 0°C, then 204mL of 25% hydrogen peroxide was added. The mixture was adjusted to pH 8 by addition of 5M sodium hydroxide and extracted with ether. The combined ether extracts were dried and concentrated. Yield: 102g.

F. Reaction of 3-(3-methoxy-4-benzyloxyphenyl)pentan-2-ol with 3,4-dimethoxybenzaldehyde to yield 4-(4-ethyl-6-methoxy-7-benzyloxy-3-methyliso-chromanyl)-1,2-dimethoxybenzene.

A solution of 46g of 3,4-dimethoxybenzaldehyde and 100g of crude 3-(3-methoxy-4-benzyloxyphenyl)pentan-2-ol in 0.3L of dioxane was saturated with hydrogen chloride gas. The mixture was heated at reflux for 3hr, then poured into water, basified with dilute sodium hydroxide and extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated.

G. Ring-opening of 4-(4-ethyl-6-methoxy-7-benzyloxy-3-methyliso-chromanyl)-1,2-dimethoxybenzene to yield 3-(4-benzyloxy-5-methoxy-2-{[3,4-dimethoxyphenyl]carbonyl}phenyl)pentan-2-one.

To a solution of 50g of crude 4-(4-ethyl-6-methoxy-7-benzyloxy-3-methyliso-chromanyl)-1,2-dimethoxybenzene in acetone at 5°C was added a solution of 50g of chromic oxide in 500mL of 35% sulfuric acid. The mixture was stirred at room temperature for 2hr, neutralized by adding cold 10% sodium hydroxide and concentrated to remove acetone. Water was added and the mixture extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated. The residue was purified by column chromatography on silica gel. Yield: 18g.

H. Debenzylation of 3-(4-benzyloxy-5-methoxy-2-{[3,4-dimethoxy-phenyl]carbonyl}phenyl)pentan-2-one to yield 3-{2-[(3,4-dimethoxy-phenyl)carbonyl]-4-hydroxy-5-methoxyphenyl}pentan-2-one.

A solution of 18g of 3-(4-benzyloxy-5-methoxy-2-{[3,4-dimethoxy-phenyl]carbonyl}phenyl)pentan-2-one in methylene chloride containing a

suspension of 2g of 10% palladium on carbon was hydrogenated at 80psi for 1hr. The mixture was filtered through diatomaceous earth and the filtrate concentrated. Yield: 15g.

I. Annulation of 3-{2-[(3,4-dimethoxy-phenyl)carbonyl]-4-hydroxy-5-methoxyphenyl}pentan-2-one by reaction with hydrazine to yield 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine.

A solution of 14g of 3-{2-[(3,4-dimethoxy-phenyl)carbonyl]-4-hydroxy-5-methoxyphenyl}pentan-2-one and 4.7mL of hydrazine in 280mL of ethanol was heated at reflux for 0.5hr. After allowing the solution to cool to room temperature, it was saturated with HCl gas. The mixture was then concentrated to a volume of about 5mL, basified with concentrated ammonium hydroxide, and extracted with methylene chloride. The combined methylene chloride extracts were dried and concentrated, and the residue recrystallized from ethyl acetate/hexane. Yield: 1.5g.

The product 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7-methoxy-8-hydroxy-5H-2,3-benzodiazepine was analyzed by HPLC, elemental analysis, GC/MS, proton NMR and differential scanning calorimetry (DSC). The data are as follows:

Purity: 98.36% by HPLC (% area). Column: Betasil Phenyl 4.6 x 150mm. Mobile Phase: Acetonitrile::0.01M Phosphate Buffer (70::30). Flow Rate: 0.5mL/min. Wavelength: 254nm.

GC-MS; M/e = 358; with the fragmentation pattern matching the proposed structure.

Differential scanning calorimetry (DSC): Temperature program 100°C to 300°C at 5°C/min, indicated molar purity = 99.14% and melting point of 146.2°C.

Elemental analysis (calculated/analysis): %C - 68.14/68.12; %H - 6.63/6.63; N - 7.43/7.20. The calculated values include 0.1M of residual ethyl acetate.

NMR ($DCCl_3$) (performed on GE QE 300): 1.08ppm (t, 3H); 1.96 (s, 3H); 2.10 (m, 2H); 2.77 (m, 1H); 3.91 (s, 3H); 3.93 (s, 3H); 3.98 (s, 3H); 5.73 (bs, 1H); 6.70 (s, 1H); 6.80 (d, 1H); 6.95 (s, 1H); 7.00 (d, 1H); 7.58 (s, 1H).

Example 5: Resolution of 1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine

The enantiomers of racemic-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine are resolved by chiral chromatography as follows.

Racemic-1-(3-hydroxy-4-methoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine is loaded onto a semipreparative (500mm x 10mm) Chirobiotic V column (ASTEC, Whippany, NJ). Elution of the enantiomeric mixture with methyl-*tert*-butyl ether/ acetonitrile (90/10 V/V), at a flow rate of 40mL/min, is monitored at 310nm. Fraction size is 10-20 mL and fractions are subjected to analytical chromatography using the same solvent composition on an analytical (150 x 4.6mm) Chirobiotic V column. The fractions containing each isolated enantiomer are processed by removing the elution solvent in vacuo.

Example 6: LTB_4 Binding Assay.

The LTB_4 receptor binding activity of racemic tofisopam and enantiomerically pure (*R*)- and (*S*)-tofisopam was determined via the guinea pig spleen membrane assay of Cheng *et al.*, *J. Pharmacol. Exp. Ther.*, 236(1), 126-132, 1986.

Reactions were carried out in a phosphate buffer (pH 7.4) containing NaCl, $MgCl_2$, EDTA, and bacitracin. The reaction volume of 150µL containing 1.0mg/mL of the Guinea pig spleen membrane preparation and 1nM [^{3}H]LTB_4, with or without a competitor, was incubated at 0-4°C for 2 hours. Competitors included 2,3-benzodiazepines, and LTB_4 as a control. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. The filter was washed with cold buffer, dried and placed in a scintillation vial. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of the test compound with the LTB_4 binding site. Data gathered in the binding experiments for test compounds and standards are

shown graphically in Fig. 1, Fig. 2 and Fig. 3, and summarized in Table 2 below.

Table 2: Summary of [^{3}H]LTB$_4$ binding data for (*R*)-, (*S*)- and racemic-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine

Test Substance	K_i (μM)
LTB$_4$	0.0002-0.0009
(*S*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine	76.0
Racemic-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine	4.52
(*R*)-1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine	0.444

The binding data shows that binding of racemic 1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine to the LTB$_4$ receptor is primarily due to the (*R*)-enantiomer, which binds with a K_i greater than 150x that of the (*S*)-enantiomer.

Example 7: Effect of Tofisopam in a Rabbit Model of LTB$_4$-induced Dermal Inflammation.

A. Test Animals and Test Compounds.

Ten female New Zealand White Rabbits were assigned to dose groups as summarized in Table 3 below.

Table 3: Test groups for the Rabbit model of LTB$_4$-induced dermal inflammation.

Test Group	Animal #	Test article	Dose (mg/kg, IP)
1	151-152	Vehicle	0
2	251-252	(*S*)-tofisopam	60
3	351-352	(*R*)-tofisopam	60
4	451-452	(*R,S*)-tofisopam	60
5	551-552	dexamethasone	0.5

Test compounds were prepared as follows. The vehicle was first prepared by dissolving 100mg of hydroxypropylmethylcellulose 2910 (HPMC)(Sigma Chemical, St. Louis, MO) in 50mL of 0.9% saline to yield a

concentration of 2% HPMC. The three test articles, (*R*)-, (*S*)- and racemic tofisopam, were formulated by adding 1g of each test article to 10mL of vehicle.

B. Dosing and Intradermal Challenge.

On Day 0, the rabbits were sedated with ketamine/xylazine (35/5 mg/kg, s.c.) and an area of approximately 8x14 cm on the back was closely and carefully clipped to expose the skin, but not inflame or otherwise damage the epithelium. A grid of ten squares each approximately 2.5 x 2.5 cm was drawn on each animal's back using an indelible marker. The animals were then dosed intraperitoneally with the appropriate test article corresponding to the group. Thirty minutes after dosing with test or control article, the animals were challenged intradermally with the LTB_4 or LTB_4-aminopropamide (LTB_4-AP, a synthetic LTB_4 agonist) in the appropriate site as shown in Table 4. Each injection site was marked with an indelible marker in order to identify the exact location of the intradermal injection site. Sixty minutes after challenge, the animals were treated again with tofisopam or control articles. The animals were sacrificed 4 hrs. after challenge, and the intradermal injection sites were excised, fixed in 10% neutral buffered formalin (NBF), and submitted for histopathology. H&E-stained sections were read by a board-certified veterinary pathologist using light microscopy.

Table 4: Intradermal injection site grid for test animals.

	Anterior		
	0	0	
	500	500	
Left	1000	1000	Right
	2000	2000	
	2000	2000	
	Posterior		

C. Necropsy.

All animals were sacrificed 4 hr after challenge. The intradermal challenge sites were excised, placed on unique cardboard squares, fixed in 10%

NBF, and submitted for histopathology. The skin sections included the epidermis and the subcutis, down to the back musculature.

D. Histopathology.

The marked skin sections were fixed for at least 48 hours on small cardboard squares. At gross trimming, three levels were cut through marked skin region in order to assure that the injection sites is brought into the plane of section. The skins were gross trimmed, processed by dehydration, embedded in paraffin, sectioned at 3-5 μm, and stained with hematoxylin and eosin.

The tissues were evaluated histopathologically via light microscopy by a board-certified veterinary pathologist. Initially, the identity of the slides was masked in order to perform the initial evaluation and rank the slides. The slides were then unmasked and there was a careful assessment of the lesions and careful comparison of the tofisopam-treated sites to the vehicle and positive control tissues. The lesions were graded based upon the degree of inflammation and the degree of edema. The severity of the inflammation and edema was keyed as follows: 0=normal; T=trace; 1=minimal; 2=mild; 3=moderate; 4=marked.

E. Histopathological Findings.

The histopathology findings are summarized in Table 5 below. Both LTB_4 and LTB_4-ap produced focal skin wheals characterized by edema of the subdermis and a brisk, dose-dependent influx of neutrophils. The inflammation was composed of numerous neutrophils attached and marginated in small vessels, perivascular inflammatory cuffs, and scattered throughout the dermis. Additionally, some inflammation and edema was present in the superficial dermis. The administration of (*R*)-tofisopam resulted in a meaningful reduction in the severity of the inflammatory cell infiltration and less edema. There were no meaningful reduction in the inflammation and edema in the vehicle-treated, the (*S*)-tofisopam treated, the racemic tofisopam treated, or the dexamethasone-treated animals.

Table 5: Summary of histologic findings in rabbits injected intradermally with LTB_4 or LTB-ap and treated with (*R*)-, (*S*)-, or racemic tofisopam, vehicle or dexamethasone.

Test animal/ injection site	LT Agent	Dose (ng)	Test compound	Inflammation score	Edema score
151/0-L	Vehicle	0	vehicle	T	T
151/0-R	Vehicle	0	vehicle	1	1
151/500-L	LTB_4	500	vehicle	1	1
151/500-R	LTB-ap	500	vehicle	1	2
151/1000-L	LTB_4	1000	vehicle	3	2
151/1000-R	LTB-ap	1000	vehicle	3+	2
151/2000-L	LTB_4	2000	vehicle	3	2
151/2000-R	LTB-ap	2000	vehicle	3	2
151/2000-L	LTB_4	2000	vehicle	3	2
151/2000-R	LTB-ap	2000	vehicle	3	2
152/0-L	Vehicle	0	vehicle	1	1
152/0-R	Vehicle	0	vehicle	1	1
152/500-L	LTB_4	500	vehicle	2	2
152/500-R	LTB-ap	500	vehicle	2	3
152/1000-L	LTB_4	1000	vehicle	3	4
152/1000-R	LTB-ap	1000	vehicle	3	4
152/2000-L	LTB_4	2000	vehicle	3	4
152/2000-R	LTB-ap	2000	vehicle	2	4
152/2000-L	LTB_4	2000	vehicle	2	2
152/2000-R	LTB-ap	2000	vehicle	2	2
251/0-L	Vehicle	0	(*S*)-tofisopam	T	T
251/0-R	Vehicle	0	(*S*)-tofisopam	1	1
251/500-L	LTB_4	500	(*S*)-tofisopam	1	2
251/500-R	LTB-ap	500	(*S*)-tofisopam	1	2
251/1000-L	LTB_4	1000	(*S*)-tofisopam	1	3
251/1000-R	LTB-ap	1000	(*S*)-tofisopam	1	3
251/2000-L	LTB_4	2000	(*S*)-tofisopam	3	2
251/2000-R	LTB-ap	2000	(*S*)-tofisopam	2	2
251/2000-L	LTB_4	2000	(*S*)-tofisopam	2	2
251/2000-R	LTB-ap	2000	(*S*)-tofisopam	2	1

Test animal/ injection site	LT Agent	Dose (ng)	Test compound	Inflammation score	Edema score
252/0-L	Vehicle	0	(*S*)-tofisopam	1	1
252/0-R	Vehicle	0	(*S*)-tofisopam	1	1
252/500-L	LTB_4	500	(*S*)-tofisopam	2	2
252/500-R	LTB-ap	500	(*S*)-tofisopam	3+	3
252/1000-L	LTB_4	1000	(*S*)-tofisopam	3+	4
252/1000-R	LTB-ap	1000	(*S*)-tofisopam	3+	4
252/2000-L	LTB_4	2000	(*S*)-tofisopam	3+	4
252/2000-R	LTB-ap	2000	(*S*)-tofisopam	3+	4
252/2000-L	LTB_4	2000	(*S*)-tofisopam	2	2
252/2000-R	LTB-ap	2000	(*S*)-tofisopam	2	2
351/0-L	Vehicle	0	(*R*)-tofisopam	0	0
351/0-R	Vehicle	0	(*R*)-tofisopam	1	1
351/500-L	LTB_4	500	(*R*)-tofisopam	1	1
351/500-R	LTB-ap	500	(*R*)-tofisopam	1	1
351/1000-L	LTB_4	1000	(*R*)-tofisopam	1	2
351/1000-R	LTB-ap	1000	(*R*)-tofisopam	1	2
351/2000-L	LTB_4	2000	(*R*)-tofisopam	1-2	2
351/2000-R	LTB-ap	2000	(*R*)-tofisopam	1	2
351/2000-L	LTB_4	2000	(*R*)-tofisopam	1	2
351/2000-R	LTB-ap	2000	(*R*)-tofisopam	1	2
352/0-L	Vehicle	0	(*R*)-tofisopam	1	2
352/0-R	Vehicle	0	(*R*)-tofisopam	1	2
352/500-L	LTB_4	500	(*R*)-tofisopam	1	3
352/500-R	LTB-ap	500	(*R*)-tofisopam	1	3
352/1000-L	LTB_4	1000	(*R*)-tofisopam	2	3
352/1000-R	LTB-ap	1000	(*R*)-tofisopam	2	3
352/2000-L	LTB_4	2000	(*R*)-tofisopam	1	3
352/2000-R	LTB-ap	2000	(*R*)-tofisopam	2	3
352/2000-L	LTB_4	2000	(*R*)-tofisopam	1	2
352/2000-R	LTB-ap	2000	(*R*)-tofisopam	1	1
451/0-L	Vehicle	0	(*R,S*)-tofisopam	1	2
451/0-R	Vehicle	0	(*R,S*)-tofisopam	1	2
451/500-L	LTB_4	500	(*R,S*)-tofisopam	2	2

Test animal/ injection site	LT Agent	Dose (ng)	Test compound	Inflammation score	Edema score
451/500-R	LTB-ap	500	(*R,S*)-tofisopam	2	2
451/1000-L	LTB_4	1000	(*R,S*)-tofisopam	3	3
451/1000-R	LTB-ap	1000	(*R,S*)-tofisopam	3	3
451/2000-L	LTB_4	2000	(*R,S*)-tofisopam	2	2
451/2000-R	LTB-ap	2000	(*R,S*)-tofisopam	2	2
451/2000-L	LTB_4	2000	(*R,S*)-tofisopam	1	1
451/2000-R	LTB-ap	2000	(*R,S*)-tofisopam	1	1
452/0-L	Vehicle	0	(*R,S*)-tofisopam	1	1
452/0-R	Vehicle	0	(*R,S*)-tofisopam	1	2
452/500-L	LTB_4	500	(*R,S*)-tofisopam	2	3
452/500-R	LTB-ap	500	(*R,S*)-tofisopam	2	3
452/1000-L	LTB_4	1000	(*R,S*)-tofisopam	2	3
452/1000-R	LTB-ap	1000	(*R,S*)-tofisopam	3	3
452/2000-L	LTB_4	2000	(*R,S*)-tofisopam	3	3
452/2000-R	LTB-ap	2000	(*R,S*)-tofisopam	2	3
452/2000-L	LTB_4	2000	(*R,S*)-tofisopam	3	2
452/2000-R	LTB-ap	2000	(*R,S*)-tofisopam	2	2
551/0-L	Vehicle	0	dexamethasone	1	0
551/0-R	Vehicle	0	dexamethasone	1	0
551/500-L	LTB_4	500	dexamethasone	3	3
551/500-R	LTB-ap	500	dexamethasone	2	3
551/1000-L	LTB_4	1000	dexamethasone	3	3
551/1000-R	LTB-ap	1000	dexamethasone	2	3
551/2000-L	LTB_4	2000	dexamethasone	2	3
551/2000-R	LTB-ap	2000	dexamethasone	2	2
551/2000-L	LTB_4	2000	dexamethasone	1	1
551/2000-R	LTB-ap	2000	dexamethasone	1	1
552/0-L	Vehicle	0	dexamethasone	0	1
552/0-R	Vehicle	0	dexamethasone	1	1
552/500-L	LTB_4	500	dexamethasone	2	2
552/500-R	LTB-ap	500	dexamethasone	3	2
552/1000-L	LTB_4	1000	dexamethasone	3	2
552/1000-R	LTB-ap	1000	dexamethasone	2	3

Test animal/ injection site	LT Agent	Dose (ng)	Test compound	Inflammation score	Edema score
552/2000-L	LTB_4	2000	dexamethasone	1+	3
552/2000-R	LTB-ap	2000	dexamethasone	2	3
552/2000-L	LTB_4	2000	dexamethasone	1	2
552/2000-R	LTB-ap	2000	dexamethasone	2	1

Example 8: Dextran Sulfate Sodium Induced Colitis: Mouse Model of IBD.

In this model of colitis, an acute inflammation of the colon was produced by administration of dextran sulfate sodium (DSS) as a 5% solution in tap water. This colitis was characterized by histological events and an influx of neutrophils, macrophages and mediators of inflammation similar to those observed with human inflammatory bowel diseases. Several drugs known to be of useful for treating IBD, such as corticosteroids and 5-ASA, have been shown to have activity in this model. The following study was conducted in accordance with protocols of Okayasu *et al.*, *Gastroenterology*, 98:694-702, 1990.

One hundred ten test animals (female, 6 week old Swiss Webster mice, 18-30g) were divided into ten groups, selected to eliminate any statistical differences in mean group weight.

Each animal was dosed daily (IP) with either a test substance or vehicle, starting on Day 0. Beginning on Day 1, acute colon inflammation was induced by the administration *ad libitum* in drinking water of dextran sulfate sodium (DSS) as a 5% solution in tap water (10mL/mouse/day for 5-6 days), with no other fluid source for animals in the DSS arm of the study. Filtered tap water was available *ad libitum* except for animals receiving 5% DSS as the sole source of fluid. After four days, signs of acute disease occurred with the loss of weight, diarrhea and bloody stools. Histological changes included initial shortening of the crypts, then areas of separation of the crypts and the *muscularis mucosae* in the absence of destructive inflammatory filtrate. After five days, pathological changes became confluent with the appearance of erosions and early

hyperplastic epithelium. Inflammation scores were high with neutrophils, lymphocytes, and plasma cells in the *lamina propria* but sparing the epithelium.

Test compounds were administered intraperitoneally (IP). Test compounds given during this period were evaluated for prophylactic activity and test compounds given after the disease state was established were evaluated for therapeutic activity. Ten test animals were assigned to each of ten dose groups listed in Table 6, below.

Table 6: Dose group assignments for the DSS-Induced Colitis: Mouse Model of IBD.

Group	Test substance	DSS or control
1	Vehicle IP daily	+ tap water
2	Vehicle IP daily	+ DSS 5% in tap water
3	Racemic tofisopam 64mg/kg IP daily	+ DSS 5% in tap water
4	Racemic tofisopam 32mg/kg IP daily	+ DSS 5% in tap water
5	Racemic tofisopam 16mg/kg IP daily	+ DSS 5% in tap water
6	(*R*)-tofisopam 64mg/kg IP daily	+ DSS 5% in tap water
7	(*R*)-tofisopam 32mg/kg IP daily	+ DSS 5% in tap water
8	(*R*)-tofisopam 16mg/kg IP daily	+ DSS 5% in tap water
9	(*S*)-tofisopam 64mg/kg IP daily	+ DSS 5% in tap water
10	(*S*)-tofisopam 32mg/kg IP daily	+ DSS 5% in tap water
11	(*S*)-tofisopam 16mg/kg IP daily	+ DSS 5% in tap water

Test animals were weighed daily from Day 0 to Day 8, or until completion of the study. The total duration of the study with DSS arm of the study was varied depending on the time progress of colitis. The condition of the test animals and consistency of stools was noted.

At the conclusion of the study, test animals were euthanized (CO_2), a midline incision was made and a stool sample was obtained. The sample was placed on a slide and tested for occult blood (Quic-Cult™, Laboratory Diagnostics Co., Morganville, NJ). Occult blood was determined by placing two drops of the reagent onto the sample and observing any color change. Occult blood presence was graded using a scoring protocol assigning a score of 0 for no color; 1 for a very light blue color (+/-) forming in > 30 seconds; 2 for a

blue color developing in 30 seconds or more (+); 3 for a change in color occurring in less than 30 seconds (++); and 4 for gross blood observable on the slide. The colon was gently stretched and the length from the colon-cecal junction to the end of the distal rectum was measured to the nearest 0.1cm. A Disease Activity Index (DAI) was determined using the criteria summarized in Table 7 below:

Table 7: Scoring criteria for determination of Disease Activity Index (DAI) in the DSS-Induced Colitis: Mouse Model of IBD.

Score	Weight loss (%)	Stool consistency	Blood in feces
0	0 or gain	Normal	Negative
1	1-4.9	Soft	+/-
2	5.0-9.9	Mixed soft and diarrhea	+
3	10-15	Diarrhea	++
4	>15	bloody diarrhea	gross blood

The scores for each test animal were added and then divided by three to give a DAI score for each animal. The data for the eleven groups is summarized in Tables 8, 9 and 10 below.

Table 8: Disease Activity Index for test animals in DSS-induced colitis study.

Test compound	n	Mean DAI ± SEM	Mean DAI* ± SEM
0.1% CMC + water	10	0.07 ± 0.04	0.00 ± 0.00
0.1% CMC + DSS	10	2.83 ± 0.24	3.25 ± 0.24
Racemic tofisopam 64mg/kg IP daily	10	2.87 ± 0.31	3.25 ± 0.26
Racemic tofisopam 32mg/kg IP daily	10	2.50 ± 0.30	2.85 ± 0.24
Racemic tofisopam 16mg/kg IP daily	9	2.59 ± 0.27	2.61 ± 0.30
(*R*)-tofisopam 64mg/kg IP daily	10	1.77 ± 0.28[1]	2.20 ± 0.26[1]
(*R*)-tofisopam 32mg/kg IP daily	10	2.37 ± 0.30	2.75 ± 0.26
(*R*)-tofisopam 16mg/kg IP daily	9	2.33 ± 0.40	2.61 ± 0.37
(*S*)-tofisopam 64mg/kg IP daily	9	2.93 ± 0.22	3.17 ± 0.19
(*S*)-tofisopam 32mg/kg IP daily	10	2.77 ± 0.26	3.15 ± 0.22
(*S*)-tofisopam 16mg/kg IP daily	10	2.37 ± 0.24	2.85 ± 0.18

[1] – Significant difference from vehicle + DSS control – Two-tailed T-test, $p < 0.05$

DAI – Disease Activity Index; DAI* - DAI without weight loss parameter.

Table 9: Colon Length assessment for test animals in DSS-induced colitis study.

Test compound	n	Mean colon length CM ± SEM	% of normal length	Colon shortening inhib. %
0.1% CMC + water	10	12.5 ± 0.11	100	--
0.1% CMC + DSS	10	7.9 ± 0.18	63.5	--
Racemic tofisopam 64mg/kg IP daily	10	8.2 ± 0.18	65.5	7
Racemic tofisopam 32mg/kg IP daily	10	8.3 ± 0.11	66.7	9
Racemic tofisopam 16mg/kg IP daily	10	8.2 ± 0.32	65.4	7
(*R*)-tofisopam 64mg/kg IP daily	10	9.8 ± 0.35	78.5	41[1]
(*R*)-tofisopam 32mg/kg IP daily	10	8.9 ± 0.41	71.3	22
(*R*)-tofisopam 16mg/kg IP daily	9	9.0 ± 0.28	72.4	24
(*S*)-tofisopam 64mg/kg IP daily	9	8.2 ± 0.20	65.4	7
(*S*)-tofisopam 32mg/kg IP daily	10	8.1 ± 0.19	64.9	4
(*S*)-tofisopam 16mg/kg IP daily	10	8.4 ± 0.19	67.3	11

[1] – Significant difference from vehicle + DSS control – $p < 0.05$ One way ANOVA and Tukey-Kramer Multiple Comparisons Test.

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Table 10: % Weight change for test animals in DSS-induced colitis study.

Test Compound	Mean weight (g) and % weight change ± SEM						
	Day 0	Day 8	% change	Day 9	% change	Day 10	% change
0.1% CMC + water	26.1±0.4	26.5±0.6	+1.5±1.8	26.8±0.6	+2.7±1.4	26.5±0.5	+1.6±1.3
0.1% CMC + DSS	26.1±0.5	25.8±0.6	-1.2±1.3	25.0±0.6	-4.3±0.9	24.1±0.6	-7.7±1.6
Racemic tofisopam 64mg/kg IP daily	26.0±0.4	25.5±0.7	-2.0±1.8	24.5±0.8	-5.8±2.3	23.5±0.9	-9.6±3.0
Racemic tofisopam 32mg/kg IP daily	26.1±0.4	25.6±0.5	-1.8±1.5	24.7±0.6	-5.3±2.0	24.0±0.7	-8.0±2.4
Racemic tofisopam 16mg/kg IP daily	25.9±0.4	25.1±0.6	-3.1±1.5	24.0±0.8	-7.4±2.5	22.5±1.0	-13.3±3.1
(*R*)-tofisopam 64mg/kg IP daily	26.2±0.5	26.2±0.5	+0.1±1.9	25.4±0.5	-2.9±1.9	25.4±0.5	-2.8±2.5
(*R*)-tofisopam 32mg/kg IP daily	25.0±0.4	26.2±0.4	+0.8±1.6	25.5±0.6	-1.9±2.1	24.6±0.6	-4.4±2.1
(*R*)-tofisopam 16mg/kg IP daily	26.1±0.5	26.1±0.6	0.0±1.4	25.2±0.6	-3.3±1.8	24.1±0.8	-7.5±3.0
(*S*)-tofisopam 64mg/kg IP daily	25.9±0.4	25.6±0.6	-1.2±1.5	24.1±0.5	-6.9±1.8	23.2±0.6	-10.4±1.7
(*S*)-tofisopam 32mg/kg IP daily	25.9±0.4	25.1±0.6	-3.0±2.3	24.6±0.6	-4.9±2.4	23.2±0.9	-10.4±3.2
(*S*)-tofisopam 16mg/kg IP daily	26.0±0.4	26.0±0.5	0.0±1.5	25.6±0.5	-1.5±1.5	24.6±0.6	-5.4±1.6

[1] – Significant difference from Vehicle + DSS group – $p < 0.05$ – One way ANOVA and Tukey-Kramer Multiple Comparisons Test. Statistical Analysis Incomplete.

The data show that at a dose of 64mg per kg, (*R*)-tofisopam provided significant protection from the LTB_4-mediated inflammatory responses to DSS-induced colitis. This assessment was based on colon length assessment and an overall Disease Activity Index (DAI) which incorporates scores for indices of colitis progression that include weight loss, stool consistency and amount of detected blood in feces.

Example 9: Dextran Sulfate Sodium Induced Colitis- Effects of Low-Dosage R-Tofisopam.

This study was conducted using a mouse model of inflammatory bowel disease as described by Okayasu et al (*Gastroenterology* 98:694-702, 1990) and modified by Murthy et al. (*Digest. Dis. Sci.* 38:1722-1734, 1993). In brief, the method involves feeding 5% dextran sulfate sodium (DSS) in drinking water for 5-6 days to mice. After four days, signs of acute disease occur with the loss of weight, diarrhea and bloody stools. Histological changes include initial shortening of the crypts, then areas of separation of the crypts and the muscularis mucosae in the absence of destructive inflammatory infiltrate. After five days, pathological changes become confluent with the appearance of erosions and early hyperplastic epithelium. Inflammation Scores are high with neutrophils, lymphocytes, and plasma cells in the lamina propria but sparing the epithelium. Agents given during this period are tested for potential prophylactic activity. Those given after the disease is established are used to evaluate therapeutic activity.

A. Experimental Design and Analysis.

Female CVF (Swiss derived) mice were obtained from Hilltop Labs (Scottdale, PA). The animals were grouped housed (5 mice/cage) in plastic cages with water absorbent bedding. The animal room was temperature controlled and maintained on a 12-hour light/dark cycle. Following an acclimation period of 13 days, 60 healthy female mice were selected for the study. The test animals were distributed (10 mice/group) into each one of the six test groups as shown below.

GROUP NUMBER	PRODUCT ID	DOSE LEVEL (mg IC/0.2 ml)
1	Vehicle (0.5% CMC in distilled water) no DSS	0
2	Vehicle (0.5% CMC in distilled water) with DSS	0
3	Mesalamine (5-ASA)	4
4	R-Tofisopam + 5% DSS	3.2
5	R-Tofisopam + 5% DSS	1.6
6	R-Tofisopam + 5% DSS	0.8

IC - intracolonic

The test compounds were suspended in a 0.5% w/w solution of carboxymethylcellulose (CMC) in distilled water. Each group of animals received the appropriate dose of the test compound starting on Day 0, as described above, by intracolonic (IC) administration using a ball tipped dosing needle and syringe at a daily dose volume of 0.2 ml/mouse/day. Groups 2 through 6 were given a 5% w/w solution of dexatran sulfate sodium (DSS) in distilled water substituted for their normal water supply from Day 1 through the day before necropsy. Group 1 animals received water bottles containing water without DSS. No other source of fluids was available. Bottles were refilled daily with the appropriate volume of fresh DSS solution or water.

All mice were weighed on Day 0 and then daily from Days 5 through 9 and observed for signs of gross toxicity and behavioral changes, consistency of stool and presence of gross blood during the study. On Day 9, all surviving animals were euthanized by CO_2 inhalation and necropsied. Following euthanasia, a stool sample was obtained from the colon of each animal and was tested for occult blood (Quik-Cult Laboratory Diagnostics Co., Morganville, NJ). The colons were then removed and the length from the colo-cecal junction to the end of the distal rectum was measured. The whole colon was collected from each animal and preserved in 10% formalin.

For each group, the disease activety index (DAI) was determined by evaluating changes in weight, Hemoccult positivity or gross bleeding, and stool consistency using the following system.

Criteria for Scoring Disease Activity Index (DAI)*

Score	Weight Loss (%)	Stool#	Blood in Feces
0	0 or gain	Normal	Negative
1	1-4.9	Soft	+/-
2	5.0-9.9	Mixed (soft & diarrhea)	+
3	10-15	Diarrhea	++
4	>15	Bloody diarrhea	Gross blood

*DAI= (combined score of weight loss, stool consistency and bleeding)/3
Normal stools = well-formed pellets; diarrhea = liquid stools that stick to the anus.

Data was analyzed using analysis of variance (single factor). Statistical significance between test and control groups was established at a probability of $p < 0.05$.

B. Results and Discussion.

In this study DSS produced a typical degree of colonic inflammation, colonic shortening and loss of body weight. Two of ten mice died in the control group. Mice were sacrificed on the ninth day. Results of the study are tabulated in Table 11A-C and Table 12 below.

The results show that R-Tofisopam produced a significant reduction in DAINWT (DAI without the weight factor) but not DAI at dosages of 3.2, 1.6 and 0.8 mg administered IC daily (Table 11A).

Referring to Table 11B, all doses of R-Tofisopam produced a statistically significant inhibition of colonic shortening by DSS, with maximal activity at 1.6 mg.

There was no statistically significant difference in weight loss (percent weight change) compared to DSS + Vehicle controls (Table 11C). Collectively the data show that low doses (in the range of about 0.5-5.0 mg per day) of R-Tofisopam are effective to significantly reduce the disease activity index (without the weight factor) and inhibit colonic shortening in a mouse model of DSS-induced colitis when R-tofisopam is applied at or near the gastrointestinal lining.

Mesalamine (5-ASA) is the active component of sulfasalazine. In contrast to results with R-Tofisopam, 4 mg/mouse of mesalamine administered by the

intracolonic route did not produce a statistically significant decrease in DAI or DAINWT (Table 11A), or protection from DSS-induced loss of body weight (Table 11C). However, mesalamine did produce a significant protection from DSS-induced colonic shortening (Table 11B).

Table 11: Effect of R-Tofisopam or Mesalamine in DSS-Induced Colitis in Mice.

A: Disease Activity Index

Compound	Dose IC mg daily	N	Mean DAI±SEM	Mean DAINWT±SEM
0.5% CMC + Water	---	10	0.10 ± 0.07**	0.05 ± 0.05**
0.5% CMC + DSS	---	8	2.54 ± 0.24	3.06 ± 0.22
Mesalamine + DSS	4	10	2.33 ± 0.16	2.40 ± 0.19
R-Tofisopam + DSS	3.2	10	2.00 ± 020	2.10 ± 0.16**
R-Tofisopam + DSS	1.6	10	1.83 ± 0.13	1.95 ± 0.23**
R-Tofisopam + DSS	0.8	8	1.96 ± 0.31	2.00 ± 0.23**

Statistical evaluation ANOVA one-way.
Statistically significant difference from vehicle + DSS control: *p<0.05; ** p<0.01
Dunnett Multiple Comparison Test – Control group = Vehicle + DSS
DAI – Disease Activity Index
DAINWT – Disease Activity Index without weight loss parameter

B: Colon Length

Compound	Dose IC mg daily	N	Mean Colon Length CM ± SEM	Percent of normal length shortening	% Inh. Colonic Length
0.5% CMC + Water	---	10	12.5 ± 0.14**	100	---
0.5% CMC + DSS	---	8	7.4 ± 0.24	59.7	---
Mesalamine + DSS	4	10	9.1 ± 0.30**	72.7	32
R-Tofisopam + DSS	3.2	10	9.1 ± 0.22**	73.1	33
R-Tofisopam + DSS	1.6	10	9.6 ± 0.22**	77.1	43
R-Tofisopam + DSS	0.8	8	9.4 ± 0.26**	75.8	40

Statistical evaluation ANOVA one-way.
Statistically significant difference from vehicle + DSS control: *p<0.05; ** p<0.01
Dunnett Multiple Comparison Test – Control group = Vehicle + DSS

Table 11 (cont.): Effect of R-Tofisopam or Mesalamine in DSS-Induced Colitis in Mice.

C: Percent Weight Change from Pre-DSS Starting

Mean Weight (grams) and Weight Change SEM

Compound	Dose mg/IC	Day 0	Day 7	% change	Day 8	% change	Day 9	% change
0.5% CMC + Water	—	28.5 ± 0.2	29.0 ± 0.3	+1.8 ± 1.2	29.2 ± 0.3	+2.5 ± 1.1	29.2 ± 0.4 **	+2.5 ± 1.3
0.5% CMC + DSS	—	27.4 ± 0.2	27.0 ± 0.6	-1.3 ± 2.3	26.8 ± 0.6	-2.2 ± 2.5	25.8 ± 0.8	-5.9 ± 2.9
Mesalamine + DSS	4	27.3 ± 0.3	26.9 ± 0.3	-1.4 ± 1.1	27.2 ± 0.4	-0.3 ± 1.0	24.7 ± 0.5	-9.4 ± 2.1
R-Tofisopam + DSS	3.2	28.8 ± 0.2	28.9 ± 0.5	+0.3 ± 1.5	27.8 ± 0.5	-3.5 ± 1.6	26.8 ± 0.6	-6.9 ± 2.0
R-Tofisopam + DSS	1.6	27.9 ± 0.3	27.9 ± 0.6	0.0 ± 1.9	27.1 ± 0.6	2.9 ± 1.9	26.2 ± 0.6	-6.1 ± 2.0
R-Tofisopam + DSS	0.8	28.4 ± 0.4	27.4 ± 0.6	-3.5 ± 2.0	26.6 ± 0.6	-0.9 ± 1.4	27.0 ± 0.7	-4.8 ± 2.3

Statistical evaluation ANOVA one-way.
Statistically significant difference from vehicle + DSS control: *$p<0.05$;
** $p<0.01$
Dunnett Multiple Comparison Test – Control group = Vehicle + DSS

Body weight data for individual mice are tabulated in Table 12. Table 12 also indicates that no deaths occurred in the groups receiving the 3.2 or 1.6 mg daily dose of R-Tofisopam; however two deaths occurred in the low dose group (0.8 mg). No deaths occurred in the Mesalamine group.

Table 12. Body weights in DSS-Induced Colitis Model

Group 1: Vehicle + Tap Water

Animal No.	Sex	Bodyweight (g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
5991	F	29	26	26	28	28	27
5992	F	28	30	30	30	30	30
5993	F	28	26	26	27	28	28
5994	F	29	28	28	29	30	30
5995	F	29	28	28	30	30	30
5996	F	28	30	30	30	30	30
5997	F	29	29	29	29	29	30
5998	F	29	29	30	30	30	30
5999	F	28	29	29	29	29	29
6000	F	28	28	28	28	28	28

Group 2: Vehicle + DSS

Animal No.	Sex	Bodyweight(g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
6001	F	27	27	27	27	28	27
6002	F	27	25	25	23	21	[1]
6003	F	27	27	26	27	26	25
6004	F	27	28	28	29	28	26
6005	F	28	27	26	24	23	21
6006	F	27	28	27	27	26	25
6007	F	28	29	29	29	28	28
6008	F	28	27	26	26	27	27
6009	F	27	28	27	[2]	-	-
6010	F	27	28	27	27	28	27

[1] Animal #6002 was found dead on Day 9 (B.W. 0.20g).

[2] Animal #6009 was found dead on Day 7 (B.W. 0.25g).

Table 12 (cont.): Bodyweights

Group 3: Mesalamine (5-ASA) (4 mg IC)

Animal No.	Sex	Bodyweight (g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
6011	F	28	30	28	29	29	26
6012	F	28	28	26	27	28	24
6013	F	28	26	25	26	27	23
6014	F	29	30	28	28	28	25
6015	F	28	28	26	27	27	23
6016	F	27	26	26	26	27	26
6017	F	27	28	28	27	28	27
6018	F	26	26	26	26	26	24
6019	F	26	26	26	26	25	23
6020	F	26	28	27	27	27	26

Group 4: R-Tofisopam (3.2 mg IC)

Animal No.	Sex	Bodyweight (g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
6021	F	28	28	28	29	28	28
6022	F	29	30	31	31	30	29
6023	F	29	30	29	31	31	30
6024	F	29	28	28	28	27	26
6025	F	29	30	29	30	28	27
6026	F	29	28	27	28	27	25
6027	F	29	31	30	29	27	25
6028	F	28	28	27	27	26	26
6029	F	28	27	26	26	26	24
6030	F	30	31	30	30	28	28

Table 12 (cont.): Bodyweights

Group 5: R-Tofisopam (1.6 mg IC)

Animal No.	Sex	Bodyweight (g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
6031	F	27	29	28	29	28	28
6032	F	29	29	28	28	28	27
6033	F	28	29	29	29	29	28
6034	F	28	28	27	28	27	27
6035	F	27	28	28	28	27	26
6036	F	27	27	25	24	23	22
6037	F	29	30	29	31	30	28
6038	F	28	28	27	28	27	26
6039	F	29	27	27	27	26	25
6040	F	27	27	26	27	26	25

Group 6: R-Tofisopam (0.8 mg IC)

Animal No.	Sex	Bodyweight (g)					
		Day 0	Day 5	Day 6	Day 7	Day 8	Day 9
6041	F	27	27	26	26	26	25
6042	F	29	[1]	-	-	-	-
6043	F	28	30	29	29	29	28
6044	F	29	30	30	30	30	29
6045	F	27	28	27	28	28	26
6046	F	29	28	28	31	31	30
6047	F	29	27	27	29	29	28
6048	F	28	24	25	27	27	25
6049	F	29	24	24			
6050	F	30	26	27	29	29	25

[1] Animal #6042 was found dead on Day 5 (B.W. 23 g).

[2] Animal #6049 was found dead on Day 7 (B.W. 24 g).

Example 10: Effects of R-Tofisopam on Arachidonic Acid –Induced Inflammation Model in Mouse Ear.

In this study the anti-inflammatory effects of R-tofisospam were tested in a model of acute inflammation of the mouse ear produced by administration of arachidonic acid Several drugs have been shown to have anti-edematous

effects in this model. The following study was conducted in accordance with the protocols of Burchart et al., *Prostaglandins Leukot. Essent. Fatty Acids* 1997 Apr; 56(4):301-306; *Prostaglandins Leukot. Essent. Fatty Acids* 1999 Jan;60(1):5-11.

A. Test Animals and Test Compounds.

Forty Swiss-Webster female mice 8-9 weeks of age, ranging in weight from 20-24g, were group housed and acclimated for six days in a controlled environment (temperature 18-26^{0}C; relative humidity 30-70%; 12 hours artificial light and 12 hours dark) in compliance with the National Research Council "Guide for the Care and Use of Laboratory Animals." Temperature and humidity were monitored daily. All animals had access to drinking water and Certified Rodent Diet (TEKLAD) *ad libitum* and were monitored at least once daily for any abnormalities or for the development of infectious disease.

Test compounds were prepared as follows. A solution of arachidonic acid (AA; Cat. No. A-9673, Sigma Chemical, St. Louis, MO) was prepared at room temperature at a concentration of 200 mg/ml in EtOH vehicle (Pharmco, Lot 0110264), transferred to an amber glass screw top vial and stored until use at approximately -20^{0}C. Test article R-tofisopam (Vela Pharmaceuticals, Lawrenceville NJ) was stored in powder form at room temperature, protected from light. Just prior to use, test article solutions were prepared in EtOH vehicle at the following concentrations: 1.0, 0.1, and 0.01 mg/ml. All dose preparations were protected from light.

Following acclimation, mice were weighed, placed in individual housing, and identified by color coding. Only mice considered suitable for use were placed in the study. Mice were selected based upon body weight and apparent good health. Mice were assigned to dose groups as summarized in Table 13 below.

Table 13. Test Groups for the Mouse Ear Model of Arachidonic Acid-Induced Inflammation.

Group	Number of Animals	Topical Treatment 1 (outer surface of each ear)	Absorption Time	Topical Treatment 2 (inner surface)		Sacrifice Time
	Female			Right Ear	Left Ear	
1	10	Vehicle[a] 10 µl	30 minutes	Ethanol 10 µl	AA[b] 10 µl	30 minutes
2	10	R-tofisopam 0.01 mg/ml	30 minutes	Ethanol 10 µl	AA 10 µl	30 minutes
3	10	R-tofisopam 0.1 mg/ml	30 minutes	Ethanol 10 µl	AA 10 µl	30 minutes
4	10	R-tofisopam 1.0 mg/ml	30 minutes	Ethanol 10 µl	AA 10 µl	30 minutes

[b]Arachidonic acid at 2 mg/ 10 µl

B. Experimental Design and Method of Analysis.

The vehicle or test article was administered topically to the outer surface of both the right and left ear in a volume of 10 µl/ear. Following a 30 minute (±5 minutes) absorption period, a 10 µl volume of AA/EtOH prepared as described above was administered topically to the inner surface of the ear. Thirty minutes (±5 minutes) after the administration of AA/EtOH, the animals were euthanized by CO_2 asphyxiation without exsanguination. Ears were measured for thickness with a Mitutoyo micrometer and edema was calculated by subtracting the thickness measurement (in mm) of the control ear (right) from that of the test ear (left). Mean edema was calculated and compared using an ANOVA followed by a Tukey HSD Multiple Comparison Test ($p \leq 0.05$)- Systat, v.9.01.

C. Results.

All animals appeared normal during the course of the study. Mean animal data from the edema studies are presented in Table 14 and individual data are presented in Table 15. Referring to Tables 14 and 15, it is seen that the topical administration of R-tofisopam at 0.01, 0.1 and 1.0 mg/ml with a 30 minute absorption time produced 30%, 26% and 45% reductions in edema, respectively, when compared to the vehicle control group. The 45% reduction was statistically significant ($p \leq 0.05$) and was considered biologically relevant, whereas the lower dose concentrations showed only slight effects of similar magnitude.

Table 14. Effect of R-Tofisopam on Arachidonic Acid-Induced Inflammation in the Mouse Ear: Mean Animal Data.

Group	Number of Animals	Topical Treatment 1 (outer surface of each ear) (30 minute absorption)	Topical Treatment (inner surface)		Sacrifice Time (minutes)	Edema[a] (mm)	% Inhibition
	Female		Right Ear	Left Ear			
1	10	Vehicle[b] 10 µl/ear	Ethanol 10 µl	AA[c] 2 mg/10 µl	30	0.128 ± 0.010	-
2	10	R-tofisopam 0.01 mg/ml, 10 µl/ear	Ethanol 10 µl	AA[c] 2 mg/10 µl	30	0.090 ± 0.015	30
3	10	R-tofisopam 0.1 mg/ml, 10 µl/ear	Ethanol 10 µl	AA[c] 2 mg/10 µl	30	0.095 ± 0.017	26
4	10	R-tofisopam 1.0 mg/ml, 10 µl/ear	Ethanol 10 µl	AA[c] 2 mg/10 µl	30	0.070* ± 0.010	45

[a] Mean change in ear thickness (L-R) ± standard error of the mean (SEM)
[b] Ethanol
[c] Arachidonic Acid
* Statistically significant ($p \leq 0.05$) decrease from vehicle - Tukey HSD Multiple Comparison Test

Table 15. Effect of R-Tofisopam on Arachidonic Acid-Induced Inflammation in the Mouse Ear: Individual Animal Data.

Group Number	Mouse Number	Topical Treatment 1 (outer surface of each ear)	Topical Treatment (inner surface)		Measurements millimeters		Edema (L-R)
			Right Ear	Left Ear	Right Ear	Left Ear	(mm)
1	1	Vehicle	Ethanol	AA	0.305	0.393	0.088
	2	10 µl	10 µl	2 mg/10 µl	0.275	0.394	0.119
	3				0.305	0.420	0.115
	4	Absorption			0.336	0.539	0.203
	5	Time			0.319	0.456	0.137
	6	30 minutes			0.285	0.418	0.133
	7				0.296	0.426	0.130
	8				0.300	0.395	0.095
	9				0.293	0.436	0.143
	10				0.289	0.409	0.120
2	11	R-tofisopam	Ethanol	AA	0.296	0.359	0.063
	12	0.01 mg/ml	10 µl	2 mg/10 µl	0.300	0.406	0.106
	13	10 µl			0.276	0.407	0.131
	14				0.325	0.355	0.030
	15	Absorption			0.350	0.426	0.076
	16	Time			0.353	0.437	0.084
	17	30 minutes			0.271	0.418	0.147
	18				0.302	0.472	0.170
	19				0.286	0.351	0.065
	20				0.334	0.360	0.026

Vehicle = EtOH

Table 15, cont'd.

Group Number	Mouse Number	Topical Treatment 1 (outer surface of each ear)	Topical Treatment (inner surface)		Measurements millimeters		Edema (L-R)
			Right Ear	Left Ear	Right Ear	Left Ear	(mm)
3	21	R-tofisopam	Ethanol	AA	0.256	0.399	0.143
	22	0.1 mg/ml	10 µl	2 mg/10 µl	0.257	0.345	0.088
	23	10 µl			0.285	0.326	0.041
	24				0.276	0.313	0.037
	25	Absorption			0.324	0.450	0.126
	26	Time			0.339	0.490	0.151
	27	30 minutes			0.283	0.371	0.088
	28				0.295	0.468	0.173
	29				0.294	0.386	0.092
	30				0.285	0.294	0.009
4	31	R-tofisopam	Ethanol	AA	0.280	0.329	0.049
	32	1.0 mg/ml	10 µl	2 mg/10 µl	0.300	0.325	0.025
	33	10 µl			0.280	0.374	0.094
	34				0.282	0.417	0.135
	35	Absorption			0.320	0.350	0.030
	36	Time			0.309	0.397	0.088
	37	30 minutes			0.273	0.359	0.086
	38				0.281	0.342	0.061
	39				0.265	0.345	0.080
	40				0.291	0.346	0.055

The data show that topical administration of R-tofisopam at 1.0 mg/ml produced a biologically relevant and statistically significant ($p \leq 0.05$) reduction in mouse ear inflammation induced by arachidonic acid.

All references cited herein are incorporated by reference. The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indication the scope of the invention.